ehp ENVIRONMENTAL HEALTH PERSPECTIVES
http://www.ehponline.org

# MicroRNAs as Potential Signatures of Environmental Exposure or Effect: A Systematic Review

**Karen Vrijens, Valentina Bollati, and Tim S. Nawrot**

http://dx.doi.org/10.1289/ehp.1408459

**Received: 22 March 2014**
**Accepted: 14 January 2015**
**Advance Publication: 16 January 2015**

# MicroRNAs as Potential Signatures of Environmental Exposure or Effect: A Systematic Review

Karen Vrijens,[1] Valentina Bollati,[2] and Tim S. Nawrot[1,3]

[1]Centre for Environmental Sciences, Hasselt University, Diepenbeek, Belgium; [2]Center of Molecular and Genetic Epidemiology, Department of Clinical Sciences and Community Health, Università degli Studi di Milano, Milan, Italy; [3]School of Public Health, Occupational and Environmental Medicine, KU Leuven, Leuven, Belgium

**Address correspondence to** Tim S. Nawrot, Centre for Environmental Sciences, Hasselt University, Agoralaan gebouw D, B-3590 Diepenbeek, Belgium. Telephone: 0032/11-26.83.82. E-mail: tim.nawrot@uhasselt.be

**Running title:** MicroRNAs as potential signatures of the exposome

**Acknowledgments:** We thank emeritus professor Harry A. Roels (Université catholique de Louvain, Brussels, Belgium) for critical discussions and reading of the manuscript. Karen Vrijens is a postdoctoral researcher of the Research Foundation – Flanders (FWO). Dr. Bollati received support from the EU Programme "Ideas" (ERC-2011-StG 282413). The funders had no role in study design, data collection, decision to publish or preparation of the manuscript.

**Competing interests**: The authors declare they have no competing financial interests.

## Abstract

**Background:** The exposome encompasses all life-course environmental exposures from the prenatal period onwards that influence health. MicroRNAs are interesting entities within this concept as markers and causation of disease. MicroRNAs are short oligonucleotide sequences that can interact with several mRNA targets.

**Objectives:** We discuss the current state of the field on microRNAs' potential as biomarkers for environmental exposure. We address miRNA signatures in response to all types of environmental exposure a human being can be exposed to; including cigarette smoke, air pollution, nanoparticles and diverse chemicals. We describe the health conditions the identified miRNAs have been reported in, i.e. cardiovascular disease, cancer and diabetes.

**Methods:** We searched the PubMed and ScienceDirect databases to identify relevant studies.

**Results:** For all exposures incorporated in this review, 27 miRNAs were differentially expressed in at least 2 independent studies. miRNAs with expression alterations associated with smoking observed in multiple studies are miR-21, miR-34b, miR-125b, miR-146a, miR-223 and miR-340 and those miRNAs observed in multiple studies with air pollution are miR-9, miR-10b, miR-21, miR-128, miR-143, miR-155, miR-222, miR-223 and miR-338. We found little overlap between *in vitro*, *in vivo* and human studies between miRNAs and exposure. We report on disease associations for those miRNAs identified in multiple studies on exposure.

**Conclusions:** MiRNA changes may be sensitive readouts of the effects of acute and chronic environmental exposure. Therefore miRNAs are valuable novel biomarkers for exposure. Further studies should elucidate the role of the mediation effect of miRNA between exposures and effect through all stages of life to provide a more accurate assessment of the consequences of miRNA changes.

## Introduction

Most common diseases result from the combined effect of genes and environmental factors and the interactions between these two. Epigenetic effects and non-coding gene products have gained research focus over the last two decades since protein-coding genes cannot account for all observed genomic effects. Here we focus on miRNAs as key regulators of health and disease. MiRNAs are endogenous, single-stranded, short non-coding RNA sequences (~22 nucleotides) that regulate gene expression at post-transcriptional level. Since the first discovery of miRNAs in *C. elegans* (Lee et al. 1993), hundreds of miRNAs in eukaryotes were identified that influence physiological processes such as development, growth, differentiation, immune reaction and adaptation to stress (van Rooij et al. 2007; Xiao et al. 2007). Diverse disease states such as cancer and heart failure are associated with distinct miRNA signatures suggesting that specific miRNA programs are activated in pathophysiological processes (Calin et al. 2005).

Recent advances in molecular biology opened the opportunity for new approaches in population based studies, in which exposures to a broad spectrum of environmental pollutants are evaluated in concert with biological systems, a concept proposed as the "exposome" (Wild 2005). From this viewpoint, miRNAs could potentially be novel biomarkers of exposure. For the purpose of this review, we focus on the response of miRNAs to environmental exposures.

### MiRNA characteristics

MiRNA-mediated gene silencing is accomplished by base pairing of the 5′ region of miRNAs with the target mRNA sequence, leading to translational repression and/or mRNA degradation (Ambros 2004). miRNAs have been paradoxically shown to up-regulate gene expression by enhancing translation under specific conditions (Vasudevan et al. 2007). The effect of miRNA

expression on gene expression is not linear, as multiple miRNAs may target the same mRNA, and the majority of mRNAs contain multiple binding sites for miRNAs, generating a highly complex regulatory network system (Saetrom et al. 2007). For details on miRNA synthesis, biogenesis and their mechanism of action see figure 1 and reviews by (Djuranovic et al. 2011) and (Murchison and Hannon 2004).

**miRNA nomenclature**

MicroRNAs are named using the "miR" prefix and a unique identifying number (e.g., miR-1, miR-2, etc.). The identifying numbers are assigned sequentially, with identical miRNAs having the same number, regardless of organism. Paralogous sequences whose mature miRNAs differ at only one or two positions are given lettered suffixes—for example, miR-10a and miR-10b. Distinct hairpin loci that give rise to identical mature miRNAs have numbered suffixes (e.g. mir-281-1 and mir-281-2). The mature sequences are designated 'miR' in the database, whereas the precursor hairpins are labeled 'mir'. The-3p and -5p suffices sometimes observed within an miR-name, refer to the arm the mature sequence comes from. For nomenclature guidelines, see (Ambros et al. 2003).

**miRNA analysis techniques suitable for large epidemiological studies**

In recent years, miRNA expression changes following exposure to environmental toxicants, even before disease onset, have gained researchers' interest. The measure of miRNAs in large epidemiological studies needs to be high throughput and sensitive enough to detect small changes in healthy subjects. At the same time, techniques need to be affordable to be conducted on large population studies. Moreover, given the complexity of phenomena induced by exposure, not fully explained by an effect on a single transcript, current research is going towards genome-

wide techniques. Another challenge is tissue specificity of miRNAs: the availability of only non-invasive samples in epidemiological studies conducted on healthy populations, limits our capability to investigate target tissues and opens important questions on the meaning of those markers in surrogate tissues. In epidemiologic research, free and exosomal miRNAs in bodily fluids are interesting study objects for their potential to serve as a proxy for tissue-specific miRNAs. A limitation of this approach is that these differ between different bodily fluids, and it is not clear what their function is. Although they hold promise as exposure biomarkers, current studies have been primarily disease-focused (reviewed in (Etheridge et al. 2011)).

Genome-wide miRNA analysis can be achieved by amplification-based (real-time quantitative PCR, qRT-PCR), hybridization-based (microarrays), and sequencing-based (next-generation sequencing (NGS)) technologies. The method to be selected depends upon the type of sample to be analyzed and the RNA preparation protocol used. qPCR is considered the gold standard because of its sensitivity, specificity, accuracy, and simple protocols. qPCR can evaluate candidate miRNA expression or array plates, evaluating a large number of miRNAs in one reaction, to OpenArray® that allows the simultaneous amplification of a very large panel of miRNAs using nanoscale volumes. A review article comparing qRT-PCR to different array-based platforms to study m(i)RNAs was recently published by (Prokopec et al. 2013).

Several miRNA microarray chip platforms are commercially available (Affymetrix GeneChip ® miRNA 3.0 array, Agilent Human microRNA Microarrays system, Exiqon miRCURY LNA™ microarray) that differ in probe design and detection stringency. The limitation of this method is the availability and stringency of probes on the chip platform that pair with miRNAs of interest. Microarrays have the advantage of being easily correlated to mRNA expression data, thus providing functional information. Furthermore, unlike all other current miRNA analysis

techniques, microarrays allow fast analysis of all miRNAs without an arbitrary pre-selection step. However, the large amount of data produced can generate false positive results and a very time consuming step of validation by qPCR is almost a must.

Finally, NGS strategies based on deep sequencing overcome some of the technical drawbacks of probe-based methodologies, especially restriction to detection of only previously known sequences (Schulte et al. 2010). As miRNAs are sequenced directly, information about sequence variations or post-transcriptional RNA editing becomes available for further analysis. The newly developed Nanostring nCounter 27 uses two sequence-specific capture probes to allow for discrimination between similar variants of a single miRNA. NGS technologies (Illumina/Solexa, GA Roche/454 GS FLX Titanium, ABI/SOLID) allow complete miRnomes to be sequenced and allow for the discovery of novel miRNAs and isoforms. In addition, Another benefit of NGS technology is that it can identify precursor and primary miRNAs as well as their mature forms. NGS will likely become the gold standard for miRNA analysis because of its ability to sequence short fragments in a high-throughput mode. The choice between these methods is a key factor in establishing the possibility of success of any epidemiological study. Each method has PROs and CONs and need to be evaluated keeping in mind the specific research.

## Methods

### Search strategy and selection criteria

To identify the relevant papers to this topic, a comprehensive search was undertaken on the PubMed and ScienceDirect databases initially using ‘microRNA’ and ‘environmental exposure’ as key terms. We did additional searches in which we replaced microRNA by ‘mir’ or ‘miRNA’ or ‘epigenetic changes’ and we substituted ‘environmental exposure’ by ‘smoking’ or ‘passive

smoking' or 'cigarette smoke' or 'air pollution' or 'nanoparticle exposure' or 'bisphenol A' or 'endocrine disruptors' or 'chemical exposure' in every possible combination. We also considered references found in our literature search and review articles. Only articles written in English were withheld. Both published and *in press* articles were incorporated in this review. The PubMed database was searched between January 1st 1980 and June 1st 2014. Articles dealing only with the description of SNPs in miRNA genes were disregarded, as were those articles dealing only with the description of miRNAs in non-mammalian species. A flowchart detailing the search strategy is shown in Figure 2. For those microRNAs differentially expressed in response to more than one personal or environmental exposure, we researched disease phenotypes correlated with them; by searching these miRs on the Human microRNA disease database (HMDD, http://202.38.126.151/hmdd/mirna/md/) and the miR2Disease database (http://www.mir2disease.org/). Results are shown in Table 1, including the direction of regulation (up or down) of the miRNA under investigation and the ensuing phenotype.

## Results

### Smoking-induced changes in miRNA expression

The most studied environmental factor in relation to epigenetics is smoking; it was among the first factors shown to affect the miRNA machinery in humans (Spira et al. 2004). Results for *in vitro* studies concerning smoking and miRs are summarized in table 2.

Izzotti analyzed miRNA expression patterns in mouse lung after exposure to passive cigarette smoke and they established life-course related miRNA expression changes by comparing miRNA expression in lungs from unexposed newborn, post-weaning and adult mice. They observed developmental-stage specific miRNA expression profiles in which miRNAs highly

expressed in newborns tended to be lower expressed in adult mice and *vice-versa*, whereas post-weaning mice represented an intermediate (Izzotti et al. 2009). Results from *in vivo* studies concerning smoking and miRs are shown in table 3.

Two studies report a comparison between mRNA and miRNA whole genome expression patterns for smokers and non-smokers (Schembri et al. 2009; Takahashi et al. 2013). The latter study showed that quitting smoking altered the plasma miRNA profiles to resemble those of non-smokers. Let-7c and miR-150 could be of importance in the initiation of smoke-induced decline of lung function, as genes that were associated with lung function impairment by GWAS are significantly enriched in binding sites for these miRs, namely STAT-3 (Qu et al. 2009) and TNFR-II (D'hulst et al. 2006).

The effect of *in utero* exposures on health in childhood and later in life is a growing area of research interest with major public health implications (Gluckman et al. 2008). An adaptive response in the fetus to *in utero* exposures can result in persistent changes into adulthood. MiRNA expression levels in placenta can affect health later in life (Maccani et al. 2011). *In utero* studies are included in table 4.

Not surprisingly, those miRNAs frequently observed down-regulated in response to smoking, have been identified as down-regulated in lung (Takamizawa et al. 2004), pancreatic (Vogt et al. 2011) and stomach (Rahman et al. 2009) cancer. Development of cardiovascular disease is associated with up-regulation of miR-206 (Shan et al. 2009) and this microRNA has significantly higher expression levels in smokers versus non-smokers. Furthermore, two miRs frequently down-regulated in association with cigarette smoke (i.e. miR-21 and miR-146a) have lowered expression levels in type II diabetes compared to healthy controls (Zampetaki et al. 2010).

Therefore, these miRNAs could contribute to the observation that smoking is an independent risk factor for type II diabetes (Cho et al. 2009). Human studies concerning smoking-induced changes of miRNA expression are summarized in table 4. Figure 3 depicts a Venn diagram displaying the common and distinct miRNAs from *in vitro*, *in vivo* and human studies on smoking-induced miRNA alterations. miR-125b and miR-21 identified in both in *vivo* and human studies, respectively, were also reported in *in vitro* studies. Furthermore, those miRNAs identified in multiple studies are indicated in bold, such as miR-34b and miR-146a.

MiRNAs with altered expression in response to environmental and/or personal exposures in at least two independent studies and their known roles in disease etiology are summarized in Table 1. miRNAs observed in association with either environmental or personal exposures are often associated with cancer; particularly breast and lung cancer and leukemia are frequently observed in table 1. Furthermore, many aberrations in the cardiovascular system are reported, such as hypertension, heart failure, myocardial infarct and atherosclerosis. It has been long known that exposures such as air pollution and smoking can cause cardiovascular disease and cancer (Pope et al. 2011); however this table argues for a causative role in disease etiology for the listed miRNAs; rather than them being merely a marker of exposure.

**Air pollution exposure and miRNA expression**

Particulate matter (PM) is a complex mixture of small particles and liquid droplets. Particle pollution is made up of a number of components, including acids, organic chemicals, metals, and soil or dust particles. The size of particles is directly linked to their potential to cause health problems (Brunekreef and Holgate 2002). Although the clinical effects of PM exposure are obvious, the underlying mechanism of disease initiation/progression is less well understood. miRNAs play a pivotal role in maintaining the lungs healthy (Nana-Sinkam et al. 2009). As the

lungs are an important target site for PM, it is suggested miRNAs could underlie the observed health effects of PM exposure. *In vitro* studies on air pollution and miRNAs are summarized in table 5.

A cohort study amongst steel plant workers determined the effect of PM exposure on miRNA expression. Blood samples were collected at the beginning of the working week ('pre-exposure'), and at the end of the working week ('post-exposure'). PM mass and metal components were measured in the plant, and correlated with miRNA expression analyses of blood samples. Urinary 8-hydroxy-2' –deoxyguanosine (8-OH-dG) levels were measured as readout of oxidative stress. Both miR-222 and miR-21 were significantly increased in post- versus pre-exposure samples. Only miR-21 expression levels were positively correlated with 8-OH-dG (Bollati et al. 2010). As it has been reported that oxidative stress induces miR-21 expression (Cheng et al. 2009); the association between 8-OH-dG and miR-21 might simply reflect the response of miR-21 to ROS production in the blood due to the PM-induced increase in oxidative stress (Bollati et al. 2010) (Table 6).

The cardiovascular anomalies observed in association with air pollution exposure are often attributed to the generation of oxidative stress (Miller et al. 2012). MiR-21 is up-regulated in response to DEP and metal-rich PM (Bollati et al. 2010; Bourdon et al. 2012) and is highly expressed in the cardiovascular system were it plays an important role in vascular cell proliferation and apoptosis and disease (reviewed in (Cheng and Zhang 2010)). Therefore, miR-21 expression could be an important mechanistic link explaining the association between air pollution exposure and cardiovascular disease.

Levanen and colleagues (Levanen et al. 2013) observed distinct miRNA expression profiles in asthmatic patients versus controls after subway exposure. Current epidemiologic studies have identified the first miRs associated with air pollution exposure, and provide a list of putative biomarkers. Table 6 summarizes the human studies on air pollution and miRs. A Venn diagram displaying the common and distinct miRNAs from *in vitro* and human studies on air pollution-induced miRNA alterations is depicted in Figure 4. As can be seen from this figure, the only miRNAs identified in both *in vitro* as well as in human studies in association with air pollution exposure are miR-10b and miR-128. Furthermore, miRNAs -9, -21, -143, -155, -222, -223 and -338 were identified in at least two independent studies on air pollution and miRNA.

**Nanoparticles**

Nanoparticles are emitted from natural and anthropic sources, and produced via nanotechnology. Fast propagation of nanotechnologies into different industries and consumer products is causing exponential growth of nano-materials production. Hence, increasing amounts of nanoparticles reach occupational settings and the indoor and outdoor environments, thus representing a potentially serious hazard to people's health (Castranova 2011; Nel et al. 2006). Nanoparticles are also able to cross cell membranes, and their interactions with biological systems are relatively unknown (Holsapple et al. 2005). Table 7 displays the studies on nanoparticle-induced changes in miRNA expression, all of which are performed in animal models.

**Chemical exposure-induced changes in miRNA**

***Formaldehyde***

Formaldehyde is an air toxic present in the atmosphere due to emission from anthropogenic and biogenic sources. 95% of inhaled formaldehyde is absorbed within the respiratory tract (Overton

et al. 2001). Formaldehyde changes gene expression patterns in nasal and lung cells (Kim et al. 2002; Li et al. 2007). As can be observed from table 1, those miRNAs reported deregulated in association with formaldehyde exposure are involved in the development of diverse tumors (breast and gastro-intestinal cancer; melanoma) as well as heart failure. Given the capability of formaldehyde to pass deep into the lung tissue and enter the systemic circulation; the link with cardiovascular disease and cancer has been widely discussed and is reviewed in (Kim KH et al. 2011). Interestingly, miR-181a, one of the miRs down-regulated after formaldehyde exposure; was reported to affect the DNA damage response in breast cancer; enabling the identification of aggressive breast tumors based on increased miR-181a expression. (Bisso et al. 2013)

***Endocrine disruptors***

Organochlorine pesticides and plasticizing agents are ubiquitous environmental endocrine disrupting compounds that impact human health. (Rubin 2011) Bisphenol A (BPA) is an industrial plasticizer often used as coating in food cans and plastic bottles. (Kang et al. 2006) Dichlorodiphenyltrichloroethane (DDT) is a well-known organochlorine pesticide. Since DDT is very persistent in the environment, accumulates in fatty tissues, and can travel long distances in the upper atmosphere, residues from historical use still remain a current threat to human health.

DDT and BPA were shown to interfere with endogenous estrogens and thyroid hormone, leading to aberrations of the reproductive, immune and central nervous system (Chevrier et al. 2013; Liu et al. 2013). DDT (Waliszewski et al. 2001) and BPA (Takahashi and Oishi 2000) cross the placental barrier and can induce *in utero* effects that could lead to detrimental effects later in life.

Prenatal exposure to BPA can alter mammary development and lead to breast cancer in humans (Soto et al. 2013). From a clinical perspective, it is interesting to note that decreased expression

of Let-7f has been associated with increased breast cancer risk (Sakurai et al. 2012) and treatment of MCF-7 breast cancer cells leads to a reduction of Let-7f expression (Tilghman et al. 2012). Furthermore, miR-146a has been proposed to induce an Alzheimer's disease pathway (Jiang et al. 2013) and is up-regulated after BPA exposure (see table 1). Therefore, the neurodegenerative consequences of BPA exposure could at least partially be attributed to miR-146a. *In vitro* studies could provide researchers with interesting miRNAs that have potential to be used as biomarkers for chemical exposure.

Polychlorinated biphenyls (PCBs) were widely used organic chemicals until their production was banned due to environmental concerns (Porta and Zumeta 2002). PCBs are stable compounds that bio-accumulate in fatty tissues (Steele et al. 1986). PCBs cause systemic changes in gene expression (Ceccatelli et al. 2006) indicating miR regulation could be involved in this process. Maternal PCB exposure leads to fetal toxicity, impaired fetal growth and pregnancy loss (Tsukimori et al. 2008).

2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a PCB that induces adverse health effects affecting the immune system (Faith and Luster 1979). Exposure of mothers during pregnancy affects the immune system of the fetus by suppressing T cell function (Camacho et al. 2004). Given the regulatory role miRs play in the immune system (Contreras and Rao 2012) it can be expected miRs are important in regulating the detrimental health effects observed after exposure to TCDD and other PCBs.

***Arsenic***

Environmental exposure to arsenic, especially the trivalent inorganic arsenic ($As^{3+}$) is a health concern given the high doses present in groundwater across the world (Fendorf et al. 2010).

Exposure to arsenic is associated with increased risk of cancer due to genomic instability (Dulout et al. 1996). Long-term exposure to arsenic induces peripheral vascular injury (Tseng 2008). A Venn diagram displaying the common and distinct miRNAs from *in vitro* and human studies on arsenic-induced miRNA alterations is depicted in Figure 5. This Figure reveals only miRNA-21 is reported in association with arsenic exposure in *in vitro* model systems and in human studies. Three miRNAs were identified in at least two independent studies on arsenic exposure and miRNA expression, namely miR-26b, -181a, and -222.

***Aluminum sulfates***

Aluminum is the most widely distributed metal in the environment and is extensively used in daily life. Chronic exposure of animals to aluminum is associated with behavioral and neuropathological changes. Epidemiological studies have shown poor performance in cognitive tests and a higher abundance of neurological symptoms for workers occupationally exposed to aluminum (Kumar and Gill 2009).

***Hexahydro-1,3,5-trinitro-s-triazine (RDX)***

The polynitramine explosive RDX is a heavily used second generation high explosive, resulting in the contamination of soils, sediments, and water (Davis et al. 2004). RDX exposure causes toxicity to the neural and immune system and increases tumor incidence for several cancers (Garcia-Reyero et al. 2011; Sweeney et al. 2012).

***Xenoestrogen diethylstilbestrol (DES)***

The synthetic estrogen diethylstilbestrol (DES) was prescribed to prevent miscarriages from the 1940s to the 1960s, to be shown later it increased breast cancer and gynecologic tumor incidence (Greenberg et al. 1984; Mittendorf 1995).

***Perfluorooctanoic acid (PFOA)***

Perfluoroalkyl chemicals are highly stable and widely used in industrialized countries. PFCs are both lipo- and hydro-phobic, and after absorption will bind to proteins in serum and liver rather than accumulate in lipids. PFOA is one of the most commonly used PFCs. Tables 8-10 report on the studies on all chemical-induced changes in miRNA expression, subdivide into *in vitro* work (table 8), *in vivo* work (table 9) and human studies (table 10).

## Conclusions

MiRNAs are omni-present in the genome and are important regulators of gene expression in response to intracellular as well as environmental cues. We discussed the response of the miRNA machinery to personal and environmental exposures, including air pollution, cigarette smoking and chemicals such as endocrine disruptors. MiRNAs have been proposed as biomarkers for disease. The literature discussed here also reveals their potential to be used as biomarkers for environmental exposure.

All miRNAs identified in different studies looking at the same environmental pollutant showed similar patterns of expression regulation. In studies were smoking-induced changes were investigated, the general observation was a down-regulation of expression. For example, miR-125b was down-regulated in response to exposure to cigarette smoke both in primary human bronchial epithelial cells (Schembri et al. 2009) as well as in mouse lung tissue. (Izzotti et al. 2009) However, when unique miRNAs had altered expression patterns in response to different environmental exposures, their direction of regulation could be the same (10/25 miRs) or opposite (15/25 miRs, 60%). The different exposures discussed in this review have their own unique health effects, so one would not expect them to have the same effect on the miRNA

machinery. However, sometimes there is also a discrepancy when looking at the same exposure indicator, for example miR-21 has been described both up- and down-regulated in response to smoking (table 4). Part of the discrepancy can be explained by the different exposure models that were used.

In general, different *in vitro* studies show little overlap, potentially due to the complex miRNA-mRNA networks that underlie the observations; and the differences in exposure utilized across studies. When studying the same environmental pollutant *in vitro*, *in vivo* or in humans, identified miRNAs are quite distinct, as can be seen from figures 3, 4 and 5. This can be partially explained by the observation that animal models not always reflect genomic responses that occur in humans (Seok et al. 2013). Discrepancy between different studies might also stem from differences in exposure duration. As was shown in a study in rats, duration of exposure influences individual miRNAs' expression pattern uniquely (Izzotti et al. 2011).

Human epidemiologic studies are necessary to observe exposure-related effects on miRNAs. Understanding the exposome requires putting together pieces of a complex puzzle. Epidemiologic studies need input from experimental studies to identify good candidate biomarkers, and *vice-versa*, results from epidemiologic studies often need follow-up by experimental studies to investigate mechanisms of action and to study tissue-dependency of effects; since such studies mostly are performed in easily accessible tissues such as blood and saliva as a surrogate for the actual target tissues.

Currently, epidemiologic studies on microRNA often involve free or exosomal miRNAs present in saliva or other bodily fluids. The matter whether these are a true reflection of the body's response and can really predict health effects. At least in blood, it has been shown that

microRNAs contained within the exsomes overlap with cellular miRNA profiles where it was observed exosomes derived from blood are enriched for miRNAs and miRNA profiles between blood cells and the cell-free exosomal fraction show important overlap (Cheng et al. 2014).

As microRNAs can regulate mRNA expression both in a negative and in a positive manner, (Vasudevan et al. 2007) and since many miRNAs can bind the same mRNA, (Saetrom et al. 2007) it is hard to draw conclusions from miRNA studies without studying the concurrent mRNA(s) expression pattern. This information is rare in the current literature on epidemiologic studies of miRNAs. These findings underscore the complex networks that are built by miRNAs and the mRNAs they regulate, since one miR can influence many mRNAs according to the timing and its pattern of expression.

One note of caution, many of the discussed studies describe large-scale microarray profiling, whilst follow-up and validation with more quantitative approaches often lags behind. This is understandable because of the cost and labor-intensity inherent to these techniques; however it is a working point to identify the miRNAs responsive to environmental exposures.

The field is currently publishing extensive lists of microRNAs responsive to environmental exposures; demonstrating their utility as biomarkers of effect. Future research should focus on establishing the molecular mechanism behind miRNA expression changes in response to exposure; to determine whether the changes in miRNA expression are merely a symptom of the (patho)physiological processes the organism undergoes after exposure, or if miRNAs are the drivers responsible for these changes. For more information on the putative mechanisms of action behind miRNAs response to environmental exposure, see recent review by (Izzotti and Pulliero 2014). Furthermore, the effect on putative mRNA targets of the identified miRNAs

should also be studied to determine whether the miRNA expression change has functional consequences, and which mRNAs are true miRNA targets under the given circumstances.

At present, not much is known on whether environmental exposures induce long-term changes in human miRNA expression, or whether these have a transient character. In order to address this problem, more longitudinal studies should be conducted looking at the long-term effects of exposure. Results from animal studies suggest that miRNA expression changes in response to formaldehyde exposure are transient and revert to normal levels after recovery from exposure. (Rager et al. 2014); although others reported miRNA profiles in target organs do not recover one week after cessation of long-term cigarette smoke exposure (Izzotti et al. 2011). In humans; miRNA expression profiles of smokers have been observed to resemble those of non-smokers when people quit smoking (Takahashi et al. 2013).

Follow-up in future generations is necessary to determine the heritability of the miRNA expression changes. More in particular, it would be very interesting to look at the effect of *in utero* environmental exposures on development of disease in later life, and the role miRs play in inducing these health effects. Furthermore, long-term longitudinal studies would also allow to distinguish between cause and effect of miRNA expression and environmental exposure. They will also allow to estimate the contribution of miRNAs to disease development. MiRNAs have proven they can be used as biomarkers of disease as well as biomarkers for environmental exposure, and hold great potential to account for disease etiology.

## References


Ambros V. 2004. The functions of animal microRNAs. Nature 431(7006): 350-355.

Ambros V, Bartel B, Bartel DP, Burge CB, Carrington JC, Chen X, et al. 2003**.** A uniform system for microRNA annotation. RNA. 9(3):277-9.

Avissar-Whiting M, Veiga KR, Uhl KM, Maccani MA, Gagne LA, Moen EL, et al. 2010. Bisphenol A exposure leads to specific microRNA alterations in placental cells. Reprod Toxicol 29(4): 401-406.

Badrnya, S., R. Baumgartner, Assinger, A. 2014. "Smoking alters circulating plasma microvesicle pattern and microRNA signatures." Thromb Haemost 112(1).

Bai S, Nasser MW, Wang B, Hsu SH, Datta J, Kutay H, et al. 2009. MicroRNA-122 inhibits tumorigenic properties of hepatocellular carcinoma cells and sensitizes these cells to sorafenib. J Biol Chem 284(46): 32015-32027.

Balansky R, Longobardi M, Ganchev G, Iltcheva M, Nedyalkov N, Atanasov P, et al. 2013. Transplacental clastogenic and epigenetic effects of gold nanoparticles in mice. Mutation research 751-752: 42-48.

Beezhold K, Liu J, Kan H, Meighan T, Castranova V, Shi X, et al. 2011. miR-190-mediated downregulation of PHLPP contributes to arsenic-induced Akt activation and carcinogenesis. Toxicol Sci 123(2): 411-420.

Bisso A, Faleschini M, Zampa F, Capaci V, de Santa J, Santarpia L et al. 2013. Oncogenic miR-181a/b affect the DNA damage response in aggressive breast cancer. Cell Cycle 12(11):1679-87.

Bleck B, Grunig G, Chiu A, Liu M, Gordon T, Kazeros A, et al. 2013. MicroRNA-375 regulation of thymic stromal lymphopoietin by diesel exhaust particles and ambient particulate matter in human bronchial epithelial cells. Journal of immunology 190(7): 3757-3763.

Bollati V, Marinelli B, Apostoli P, Bonzini M, Nordio F, Hoxha M, et al. 2010. Exposure to metal-rich particulate matter modifies the expression of candidate microRNAs in peripheral blood leukocytes. Environ Health Perspect 118(6): 763-768.

Bourdon JA, Saber AT, Halappanavar S, Jackson PA, Wu D, Hougaard KS, et al. 2012. Carbon black nanoparticle intratracheal installation results in large and sustained changes in the expression of miR-135b in mouse lung. Environmental and molecular mutagenesis 53(6): 462-468.

Brunekreef B, Holgate ST. 2002. Air pollution and health. Lancet 360:1233–1242.

Busk PK, Cirera S. 2010. MicroRNA profiling in early hypertrophic growth of the left ventricle in rats. Biochem Biophys Res Commun 396(4): 989-993.

Calin GA, Ferracin M, Cimmino A, Di Leva G, Shimizu M, Wojcik SE, et al. 2005. A MicroRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. N Engl J Med 353(17): 1793-1801.

Camacho IA, Nagarkatti M, Nagarkatti PS. 2004. Effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on maternal immune response during pregnancy. Arch Toxicol 78(5): 290-300.

Caramuta S, Egyhazi S, Rodolfo M, Witten D, Hansson J, Larsson C, et al. 2010. MicroRNA expression profiles associated with mutational status and survival in malignant melanoma. J Invest Dermatol 130(8): 2062-2070.

Castranova V. 2011. Overview of current toxicological knowledge of engineered nanoparticles. J Occup Environ Med 53(6 Suppl): S14-17.

Ceccatelli R, Faass O, Schlumpf M, Lichtensteiger W. 2006. Gene expression and estrogen sensitivity in rat uterus after developmental exposure to the polybrominated diphenylether PBDE 99 and PCB. Toxicology 220(2-3): 104-116.

Chang S, Wang RH, Akagi K, Kim KA, Martin BK, Cavallone L et al. 2011. Tumor suppressor BRCA1 epigenetically controls oncogenic microRNA-155. Nat Med 17(10): 1275-1282.

Cheng Y, Liu X, Zhang S, Lin Y, Yang J, Zhang C. 2009. MicroRNA-21 protects against the H2O2-induced injury on cardiac myocytes via its target gene PDCD4. J Mol Cell Cardiol. 47(1): 5–14.

Cheng L, Sharples RA, Scicluna BJ, Hill AF. 2014. Exosomes provide a protective and enriched source of miRNA for biomarker profiling compared to intracellular and cell-free blood**.** J Extracell Vesicles: 26;3.

Cheng Y, Zhang C. 2010. MicroRNA-21 in cardiovascular disease. J Cardiovasc Transl Res 3(3): 251-255.

Chevrier J, Gunier RB, Bradman A, Holland NT, Calafat AM, Eskenazi B, et al. 2013. Maternal urinary bisphenol a during pregnancy and maternal and neonatal thyroid function in the CHAMACOS study. Environ Health Perspect 121(1): 138-144.

Cho NH, Chan JC, Jang HC, Lim S, Kim HL, Choi SH. 2009. Cigarette smoking is an independent risk factor for type 2 diabetes: a four-year community-based prospective study. Clin Endocrinol (Oxf) 71(5): 679-685.

Clay CC, Maniar-Hew K, Gerriets JE, Wang, TT, Postlethwait, EM, Evans MJ et al. 2014. Early life ozone exposure results in dysregulated innate immune function and altered microRNA expression in airway epithelium. PLoS One 9(3): e90401

Contreras J, Rao DS. 2012. MicroRNAs in inflammation and immune responses. Leukemia 26(3): 404-413.

D'hulst AI, Bracke KR, Maes T, De Bleecker JL, Pauwels RA, Joos GF, et al. 2006. Role of tumour necrosis factor-a receptor P75 in cigarette smoke–induced pulmonary inflammation and emphysema.Eur Respir J 28:102–112.

Dai Y, Sui W, Lan H, Yan Q, Huang H, Huang Y. 2009. Comprehensive analysis of microRNA expression patterns in renal biopsies of lupus nephritis patients. Rheumatol Int 29(7): 749-754.

Davis JL, Wani AH, O'Neal BR, Hansen LD. 2004. RDX biodegradation column study: comparison of electron donors for biologically induced reductive transformation in groundwater. J Hazard Mater 112(1-2): 45-54.

Deng Q, Huang S, Zhang X, Zhang W, Feng J, Wang T et al. 2014. Plasma microRNA Expression and Micronuclei Frequency in Workers Exposed to Polycyclic Aromatic Hydrocarbons. Environ Health Perspect.

Djuranovic S, Nahvi A, Green R. 2011. A parsimonious model for gene regulation by miRNAs. Science. 331(6017): 550-3.

Dulout FN, Grillo CA, Seoane AI, Maderna CR, Nilsson R, Vahter M, et al. 1996. Chromosomal aberrations in peripheral blood lymphocytes from native Andean women and children from northwestern Argentina exposed to arsenic in drinking water. Mutat Res 370(3-4): 151-158.

Earle JS, Luthra R, Romans A, Abraham R, Ensor J, Yao H, et al. 2010. Association of microRNA expression with microsatellite instability status in colorectal adenocarcinoma. J Mol Diagn 12(4): 433-440.

Etheridge A, Lee I, Hood L, Galas D, Wang K. 2011. Extracellular microRNA: a new source of biomarkers. Mutat Res 717(1-2): 85-90.

Eyholzer, M., S. Schmid, et al. 2010. "Complexity of miR-223 regulation by CEBPA in human AML." Leuk Res 34(5): 672-676.

Faith RE, Luster MI. 1979. Investigations on the effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on parameters of various immune functions. Ann N Y Acad Sci 320: 564-571.

Feitelson MA, Lee J. 2007. Hepatitis B virus integration, fragile sites, and hepatocarcinogenesis. Cancer Lett 252(2): 157-170.

Fendorf S, Michael HA, van Geen A. 2010. Spatial and temporal variations of groundwater arsenic in South and Southeast Asia. Science 328(5982): 1123-1127.

Ferretti E, De Smaele E, Po A, Di Marcotullio L, Tosi E, Espinola MS, et al. 2009. MicroRNA profiling in human medulloblastoma. Int J Cancer 124(3): 568-577.

Fossati S, Baccarelli A, Zanobetti A, Hoxha M, Vokonas PS, Wright RO et al. 2014. Ambient particulate air pollution and microRNAs in elderly men. Epidemiology 25(1): 68-78.

Fry RC, Rager JE, Bauer R, Sebastian E Peden DB, Jaspers I et al. 2014. Air toxics and epigenetic effects: ozone altered microRNAs in the sputum of human subjects. Am J Physiol Lung Cell Mol Physiol 306(12): L1129-1137.

Gambardella S, Rinaldi F, Lepore SM, Viola A, Loro E, Angelini C, et al. 2010. Overexpression of microRNA-206 in the skeletal muscle from myotonic dystrophy type 1 patients. J Transl Med 8: 48.

Gao W, Yu Y, Cao H, Shen H, Li X, Pan S, et al. 2010. Deregulated expression of miR-21, miR-143 and miR-181a in non small cell lung cancer is related to clinicopathologic characteristics or patient prognosis. Biomed Pharmacother 64(6): 399-408.

Gao W, He HW, Wang ZM, Zhao H, Lian XQ, Wang YS, et al. 2012. Plasma levels of lipometabolism-related miR-122 and miR-370 are increased in patients with hyperlipidemia and associated with coronary artery disease. Lipids Health Dis 11: 55.

Garcia-Reyero N, Habib T, Pirooznia M, Gust KA, Gong P, Warner C, et al. 2011. Conserved toxic responses across divergent phylogenetic lineages: a meta-analysis of the neurotoxic effects of RDX among multiple species using toxicogenomics. Ecotoxicology 20(3): 580-594.

Genovesi LA, Carter KW, Gottardo NG, Giles KM, Dallas PB. 2011. Integrated analysis of miRNA and mRNA expression in childhood medulloblastoma compared with neural stem cells. PloS one 6(9): e23935.

Gluckman PD, Hanson MA, Cooper C, Thornburg KL. 2008. Effect of in utero and early-life conditions on adult health and disease. N Engl J Med 359(1): 61-73.

Graff JW, Powers LS, Dickson AM, Kim J, Reisetter AC, Hassan IH, et al. 2012. Cigarette smoking decreases global microRNA expression in human alveolar macrophages. PloS one 7(8): e44066.

Greco, S.Fasanaro, P.Castelvecchio, S.D'Alessandra, Y.Arcelli, D.Di Donato, M. et al. 2012. MicroRNA dysregulation in diabetic ischemic heart failure patients. Diabetes 61(6): 1633-1641.

Greenberg ER, Barnes AB, Resseguie L, Barrett JA, Burnside S, Lanza LL, et al. 1984. Breast cancer in mothers given diethylstilbestrol in pregnancy. N Engl J Med 311(22): 1393-1398.

Guida M, Marra M, Zullo F, Guida M, Trifuoggi M, Biffali E, et al. 2013. Association between exposure to dioxin-like polychlorinated biphenyls and miR-191 expression in human peripheral blood mononuclear cells. Mutat Res 753(1): 36-41.

Halappanavar S, Nikota J, Wu D, Williams A, Yauk CL, Stampfli M. 2013. IL-1 receptor regulates microRNA-135b expression in a negative feedback mechanism during cigarette smoke-induced inflammation. J Immunol 190(7): 3679-3686.

Henke JI, Goergen D, Zheng J, Song Y, Schuttler CG, Fehr C, et al. 2008. microRNA-122 stimulates translation of hepatitis C virus RNA. EMBO J 27(24): 3300-3310.

Herberth G, Bauer M, Gasch M, Hinz D, Roder S, Olek S, et al. 2013. Maternal and cord blood miR-223 expression associates with prenatal tobacco smoke exposure and low regulatory T-cell numbers. J Allergy Clin Immunol.

Holsapple MP, Farland WH, Landry TD, Monteiro-Riviere NA, Carter JM, Walker NJ, et al. 2005. Research strategies for safety evaluation of nanomaterials, part II: toxicological and safety evaluation of nanomaterials, current challenges and data needs. Toxicol Sci 88(1): 12-17.

Hu Y, Li P, Hao S, Liu L, Zhao J, Hou Y. 2009. Differential expression of microRNAs in the placentae of Chinese patients with severe pre-eclampsia. Clin Chem Lab Med 47(8): 923-929.

Huang Y, Dai Y, Zhang J, Wang C, Li D, Cheng J, et al. 2012. Circulating microRNAs as potential biomarkers for smoking-related interstitial fibrosis. Biomarkers 17(5): 435-440.

Hulsmans M, Sinnaeve P, Van der Schueren B, Mathieu C, Janssens S, Holvoet P. 2012. Decreased miR-181a expression in monocytes of obese patients is associated with the occurrence of metabolic syndrome and coronary artery disease. J Clin Endocrinol Metab 97(7): E1213-1218.

Izzotti A, Calin GA, Steele VE, Croce CM, De Flora S. 2009. Relationships of microRNA expression in mouse lung with age and exposure to cigarette smoke and light. FASEB J 23(9): 3243-3250.

Izzotti A, Larghero P, Longobardi M, Cartiglia C, Camoirano A, Steele VE, et al. 2011. Dose-responsiveness and persistence of microRNA expression alterations induced by cigarette smoke in mouse lung. Mutat Res 717(1-2): 9-16.

Izzotti A and Pulliero A. 2014. The effects of environmental chemical carcinogens on the microRNA machinery. Int J Hyg Environ Health.

Jardim MJ, Fry RC, Jaspers I, Dailey L, Diaz-Sanchez D. 2009. Disruption of microRNA expression in human airway cells by diesel exhaust particles is linked to tumorigenesis-associated pathways. Environ Health Perspect 117(11): 1745-1751.

Ji R, Cheng Y, Yue J, Yang J, Liu X, Chen H, et al. 2007. MicroRNA expression signature and antisense-mediated depletion reveal an essential role of MicroRNA in vascular neointimal lesion formation. Circ Res 100(11): 1579-1588.

Jiang W, Zhang Y, Meng F, Lian B, Chen X, Yu X, et al. 2013. Identification of active transcription factor and miRNA regulatory pathways in Alzheimer's disease. Bioinformatics 29(20):2596-602.

Jin L, Hu WL, Jiang CC, Wang JX, Han CC, Chu P, et al. 2011. MicroRNA-149*, a p53-responsive microRNA, functions as an oncogenic regulator in human melanoma. PNAS 108(38): 15840-15845.

Ju X, Li D, Shi Q, Hou H, Sun N, Shen B. 2009. Differential microRNA expression in childhood B-cell precursor acute lymphoblastic leukemia. Pediatric hematology and oncology 26(1): 1-10.

Kang JH, Kondo F, Katayama Y. 2006. Human exposure to bisphenol A. Toxicology 226(2-3): 79-89.

Kim KH, Jahan SA, Kabir E. 2011. A review of diseases associated with household air pollution due to the use of biomass fuels. 1. J Hazard Mater. 192(2):425-31.

Kim K, Lee HC, Park JL, Kim M, Kim SY, Noh SM, et al. 2011. Epigenetic regulation of microRNA-10b and targeting of oncogenic MAPRE1 in gastric cancer. Epigenetics 6(6): 740-751.

Kim WJ, Terada N, Nomura T, Takahashi R, Lee SD, Park JH, et al. 2002. Effect of formaldehyde on the expression of adhesion molecules in nasal microvascular endothelial cells: the role of formaldehyde in the pathogenesis of sick building syndrome. Clin Exp Allergy 32(2): 287-295.

Kin, K. Miyagawa, S. Fukushima, S. Shirakawa, Y. Torikai, K. Shimamura, K., et al. 2012. Tissue- and plasma-specific MicroRNA signatures for atherosclerotic abdominal aortic aneurysm. J Am Heart Assoc 1(5): e000745.

Kong W, He L, Richards EJ, Challa S, Xu CX, Permuth-Wey J, et al. 2014. Upregulation of miRNA-155 promotes tumour angiogenesis by targeting VHL and is associated with poor prognosis and triple-negative breast cancer. Oncogene 33(6): 679-689.

Kong AP, Xiao K, Choi KC, Wang G, Chan MH, Ho CS, et al. 2012. Associations between microRNA (miR-21, 126, 155 and 221), albuminuria and heavy metals in Hong Kong Chinese adolescents. Clin Chim Acta 413(13-14): 1053-1057.

Kumar V, Gill KD. 2009. Aluminium neurotoxicity: neurobehavioural and oxidative aspects. Arch Toxicol 83(11): 965-978.

Lau P, de Strooper B. 2010. Dysregulated microRNAs in neurodegenerative disorders. Semin Cell Dev Biol 21(7): 768-773.

Lee RC, Feinbaum RL, Ambros V. 1993. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 75(5): 843-854.

Leucci, E, Zriwil, A, Gregersen LH, Jensen KT, Obad S Bellan C et al. 2012. Inhibition of miR-9 de-represses HuR and DICER1 and impairs Hodgkin lymphoma tumour outgrowth in vivo. Oncogene 31(49): 5081-5089.

Levanen B, Bhakta NR, Torregrosa Paredes P, Barbeau R, Hiltbrunner S, Pollack JL, et al. 2013. Altered microRNA profiles in bronchoalveolar lavage fluid exosomes in asthmatic patients. The Journal of allergy and clinical immunology 131(3): 894-903.

Li GY, Lee HY, Shin HS, Kim HY, Lim CH, Lee BH. 2007. Identification of gene markers for formaldehyde exposure in humans. Environ Health Perspect 115(10): 1460-1466.

Li X, Liu Y, Song G, Liu N. 2012a. A microarray for microRNA profiling in spermatozoa from adult men living in an environmentally polluted site. Bull Environ Contam Toxicol. 89(6):1111-4

Li X, Shi Y, Wei Y, Ma X, Li Y, Li R. 2012b. Altered expression profiles of microRNAs upon arsenic exposure of human umbilical vein endothelial cells. Environ Toxicol Pharmacol 34. 381-387.

Liang P, Lv C, Jiang B, Long X, Zhang P, Zhang M, et al. 2012. MicroRNA profiling in denatured dermis of deep burn patients. Burns 38(4): 534-540.

Liu C, Duan W, Li R, Xu S, Zhang L, Chen C, et al. 2013. Exposure to bisphenol A disrupts meiotic progression during spermatogenesis in adult rats through estrogen-like activity. Cell Death Dis 4: e676.

Liu XX, Li XJ, Zhang B, Liang YJ, Zhou CX, Cao DX, et al. 2011. MicroRNA-26b is underexpressed in human breast cancer and induces cell apoptosis by targeting SLC7A11. FEBS Lett 585(9): 1363-1367.

Lukiw WJ, Pogue AI. 2007. Induction of specific micro RNA (miRNA) species by ROS-generating metal sulfates in primary human brain cells. J Inorg Biochem 101(9): 1265-1269.

Lukiw WJ, Dua P, Pogue AI, Eicken C, Hill JM. 2011. Upregulation of micro RNA-146a (miRNA-146a), a marker for inflammatory neurodegeneration, in sporadic Creutzfeldt-Jakob disease (sCJD) and Gerstmann-Straussler-Scheinker (GSS) syndrome. J Toxicol Environ Health A 74(22-24): 1460-1468.

Lukiw WJ, Alexandrov PN. 2012. Regulation of complement factor H (CFH) by multiple miRNAs in Alzheimer's disease (AD) brain. Mol Neurobiol 46(1): 11-19.

Lv SQ, Kim YH, Giulio F, Shalaby T, Nobusawa S, Yang H, et al. 2012. Genetic alterations in microRNAs in medulloblastomas. Brain Pathol 22(2): 230-239.

Ma L, Young J, Prabhala, Pan E, Mestdagh P, Muth D et al. 2010. miR-9, a MYC/MYCN-activated microRNA, regulates E-cadherin and cancer metastasis. Nat Cell Biol 12(3): 247-256.

Maccani MA, Avissar-Whiting M, Banister CE, McGonnigal B, Padbury JF, Marsit CJ. 2010. Maternal cigarette smoking during pregnancy is associated with downregulation of miR-16, miR-21, and miR-146a in the placenta. Epigenetics 5(7): 583-589.

Maccani MA, Padbury JF, Marsit CJ. 2011. miR-16 and miR-21 expression in the placenta is associated with fetal growth. PLoS One 6(6): e21210.

Marcucci G, Radmacher MD, Maharry K, Mrozek K, Ruppert AS, Paschka P, et al. 2008. MicroRNA expression in cytogenetically normal acute myeloid leukemia. N Engl J Med 358(18): 1919-1928.

Marczylo EL, Amoako AA, Konje JC, Gant TW, Marczylo TH. 2012. Smoking induces differential miRNA expression in human spermatozoa: a potential transgenerational epigenetic concern? Epigenetics. 7(5):432-9.

Miller MR, Shaw CA, Langrish JP. 2012. From particles to patients: oxidative stress and the cardiovascular effects of air pollution. Future Cardiol 8(4): 577-602.

Miller TE, Ghoshal K, Ramaswamy B, Roy S, Datta J, Shapiro CL, et al. 2008. MicroRNA-221/222 confers tamoxifen resistance in breast cancer by targeting p27Kip1. J Biol Chem 283(44): 29897-29903.

Mittendorf R. 1995. Teratogen update: carcinogenesis and teratogenesis associated with exposure to diethylstilbestrol (DES) in utero. Teratology 51(6): 435-445.

Motta V, Angelici L, Nordio F, Bollati V, Fossati S, Frascati F, et al. 2013. Integrative analysis of miRNA and inflammatory gene expression after acute particulate matter exposure. Toxicol Sci. 132(2) 307-316.

Murchison EP, Hannon GJ. 2004. miRNAs on the move: miRNA biogenesis and the RNAi machinery. Curr Opin Cell Biol: 16(3):223-9.

Nagano T, Higashisaka K, Kunieda A, Iwahara Y, Tanaka K, Nagano K et al. 2013. Liver-specific microRNAs as biomarkers of nanomaterial-induced liver damage. Nanotechnology 24(40): 405102.

Nagel R, le Sage C, Diosdado B, van der Waal M, Oude Vrielink JA, Bolijn A, et al. 2008. Regulation of the adenomatous polyposis coli gene by the miR-135 family in colorectal cancer. Cancer Res 68(14): 5795-5802.

Nakanishi H, Taccioli C, Palatini J, Fernandez-Cymering C, Cui R, Kim T, et al. 2013. Loss of miR-125b-1 contributes to head and neck cancer development by dysregulating TACSTD2 and MAPK pathway. Oncogene.33(6): 702-712.

Nana-Sinkam SP, Hunter MG, Nuovo GJ, Schmittgen TD, Gelinas R, Galas D, et al. 2009. Integrating the MicroRNome into the study of lung disease. Am J Respir Crit Care Med 179(1): 4-10.

Nel A, Xia T, Madler L, Li N. 2006. Toxic potential of materials at the nanolevel. Science 311(5761): 622-627.

Ohlsson Teague EM, Van der Hoek KH, Van der Hoek MB, Perry N, Wagaarachchi P, Robertson SA, et al. 2009. MicroRNA-regulated pathways associated with endometriosis. Mol Endocrinol 23(2): 265-275.

Ota D, Mimori K, Yokobori T, Iwatsuki M, Kataoka A, Masuda N, et al. 2011. Identification of recurrence-related microRNAs in the bone marrow of breast cancer patients. Int J Oncol 38(4): 955-962.

Overton JH, Kimbell JS, Miller FJ. 2001. Dosimetry modeling of inhaled formaldehyde: the human respiratory tract. Toxicol Sci 64(1): 122-134.

Pallante P, Visone R, Ferracin M, Ferraro A, Berlingieri MT, Troncone G, et al. 2006. MicroRNA deregulation in human thyroid papillary carcinomas. Endocr Relat Cancer 13(2): 497-508.

Palma CA, Al Sheikha D, Lim TK, Bryant A, Vu TT, Jayaswal V et al. 2014. MicroRNA-155 as an inducer of apoptosis and cell differentiation in Acute Myeloid Leukaemia. Mol Cancer 13(1): 79

Perkins DO, Jeffries CD, Jarskog LF, Thomson JM, Woods K, Newman MA, et al. 2007. microRNA expression in the prefrontal cortex of individuals with schizophrenia and schizoaffective disorder. Genome Biol 8(2): R27.

Pogue AI, Li YY, Cui JG, Zhao Y, Kruck TP, Percy ME, et al. 2009. Characterization of an NF-kappaB-regulated, miRNA-146a-mediated down-regulation of complement factor H (CFH) in metal-sulfate-stressed human brain cells. J Inorg Biochem 103(11): 1591-1595.

Polikepahad S, Knight JM, Naghavi AO, Oplt T, Creighton CJ, Shaw C, et al. 2010. Proinflammatory role for let-7 microRNAS in experimental asthma. J Biol Chem 285(39): 30139-30149.

Pope CA 3rd, Burnett RT, Turner MC, Cohen A, Krewski D, Jerrett M, et al. 2011.. Lung cancer and cardiovascular disease mortality associated with ambient air pollution and cigarette smoke: shape of the exposure-response relationships. Environ Health Perspect. 2011;119(11):1616-21.

Porta M, Zumeta E. 2002. Implementing the Stockholm Treaty on Persistent Organic Pollutants. Occup Environ Med 59(10): 651-652.

Prokopec SD, Watson JD, Waggott DM, Smith AB, Wu AH, Okey AB, et al. 2013. Systematic evaluation of medium-throughput mRNA abundance platforms. RNA. 19(1):51-62.

Qian ZR, Asa SL, Siomi H, Siomi MC, Yoshimoto K, Yamada S, et al. 2009. Overexpression of HMGA2 relates to reduction of the let-7 and its relationship to clinicopathological features in pituitary adenomas. Mod Pathol 22(3): 431-441.

Qu P, Roberts J, Li Y, Albrecht M, Cummings OW, Eble JN, et al. 2009. Stat3 downstream genes serve as biomarkers in human lung carcinomas and chronic obstructive pulmonary disease. Lung Cancer 63:341–347.

Rager JE, Moeller BC, Doyle-Eisele M, Kracko D, Swenberg JA, Fry RC. 2013. Formaldehyde and Epigenetic Alterations: MicroRNA Changes in the Nasal Epithelium of Nonhuman Primates. Environ Health Perspect 121(3): 339-344.

Rager JE, Moeller BC, Miller SK, Kracko D, Doyle-Eisele M, Swenberg JA et al. 2014. Formaldehyde-associated changes in microRNAs: tissue and temporal specificity in the rat nose, white blood cells, and bone marrow. Toxicol Sci. 138(1):36-46.

Rager JE, Smeester L, Jaspers I, Sexton KG, Fry RC. 2011. Epigenetic changes induced by air toxics: formaldehyde exposure alters miRNA expression profiles in human lung cells. Environ Health Perspect 119(4): 494-500.

Rahman MM, Qian ZR, Wang EL, Sultana R, Kudo E, Nakasono M, et al. 2009. Frequent overexpression of HMGA1 and 2 in gastroenteropancreatic neuroendocrine tumours and its relationship to let-7 downregulation. Br J Cancer 100(3): 501-510.

Raitoharju E, Lyytikainen LP, Levula M, Oksala N, Mennander A, Tarkka M, et al. 2011. miR-21, miR-210, miR-34a, and miR-146a/b are up-regulated in human atherosclerotic plaques in the Tampere Vascular Study. Atherosclerosis 219(1): 211-217.

Ralfkiaer U, Hagedorn PH, Bangsgaard N, Lovendorf MB, Ahler CB, Svensson L, et al. 2011. Diagnostic microRNA profiling in cutaneous T-cell lymphoma (CTCL). Blood 118(22): 5891-5900.

Rubin BS. 2011. Bisphenol A: an endocrine disruptor with widespread exposure and multiple effects. J Steroid Biochem Mol Biol 127(1-2): 27-34.

Saetrom P, Heale BS, Snove O, Jr., Aagaard L, Alluin J, Rossi JJ. 2007. Distance constraints between microRNA target sites dictate efficacy and cooperativity. Nucleic Acids Res. 35: 2333–2342.

Sakurai M, Miki Y, Masuda M, Hata S, Shibahara Y, Hirakawa H, et al. 2012. LIN28: a regulator of tumor-suppressing activity of let-7 microRNA in human breast cancer. J Steroid Biochem Mol Biol 131(3-5): 101-106.

Scapoli L, Palmieri A, Lo Muzio L, Pezzetti F, Rubini C, Girardi A, et al. 2010. MicroRNA expression profiling of oral carcinoma identifies new markers of tumor progression. Int J Immunopathol Pharmacol 23(4): 1229-1234.

Schembri F, Sridhar S, Perdomo C, Gustafson AM, Zhang X, Ergun A, et al. 2009. MicroRNAs as modulators of smoking-induced gene expression changes in human airway epithelium. Proc Natl Acad Sci U S A 106(7): 2319-2324.

Schulte JH, Marschall T, Martin M, Rosenstiel P, Mestdagh P, Schlierf S et al. 2010. Deep sequencing reveals differential expression of microRNAs in favorabel versus unfavorable neuroblastoma. Nucleic Acids Research 38(17): 5919-5928.

Seok J, Warren HS, Cuenca AG, Mindrinos MN, Baker HV, Xu W, Richards DR, et al. 2013. Genomic responses in mouse models poorly mimic human inflammatory diseases. Proc Natl Acad Sci U S A. 110(9):3507-12

Shan ZX, Lin QX, Fu YH, Deng CY, Zhou ZL, Zhu JN, et al. 2009. Upregulated expression of miR-1/miR-206 in a rat model of myocardial infarction. Biochem Biophys Res Commun 381(4): 597-601.

Shi L, Cheng Z, Zhang J, Li R, Zhao P, Fu Z, et al. 2008. hsa-mir-181a and hsa-mir-181b function as tumor suppressors in human glioma cells. Brain Res 1236: 185-193.

Singh NP, Singh UP, Guan H, Nagarkatti P, Nagarkatti M. 2012. Prenatal exposure to TCDD triggers significant modulation of microRNA expression profile in the thymus that affects consequent gene expression. PLoS One 7(9): e45054.

Song MK, Park YK, Ryu JC. 2013. Polycyclic aromatic hydrocarbon (PAH)-mediated upregulation of hepatic microRNA-181 family promotes cancer cell migration by targeting MAPK phosphatase-5, regulating the activation of p38 MAPK. Toxicol Appl Pharmacol 273(1): 130-139.

Soto AM, Brisken C, Schaeberle C, Sonnenschein C. 2013. Does cancer start in the womb? altered mammary gland development and predisposition to breast cancer due to in utero exposure to endocrine disruptors. J Mammary Gland Biol Neoplasia 18(2): 199-208.

Spira A, Beane J, Shah V, Liu G, Schembri F, Yang X, et al. 2004. Effects of cigarette smoke on the human airway epithelial cell transcriptome. Proc Natl Acad Sci U S A 101(27): 10143-10148.

Stanitz E, Juhasz K, Toth C, Gombos K, Natali PG, Ember I. 2013. Evaluation of MicroRNA expression pattern of gastric adenocarcinoma associated with socioeconomic, environmental and lifestyle factors in northwestern Hungary. Anticancer research 33(8): 3195-3200.

Steele G, Stehr-Green P, Welty E. 1986. Estimates of the biologic half-life of polychlorinated biphenyls in human serum. N Engl J Med 314(14): 926-927.

Sturchio E, Colombo T, Boccia P, Carucci N, Meconi C, Minoia C et al. 2014. Arsenic exposure triggers a shift in microRNA expression. Sci Total Environ 472: 672-680.

Sweeney LM, Okolica MR, Gut CP, Jr., Gargas ML. 2012. Cancer mode of action, weight of evidence, and proposed cancer reference value for hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). Regul Toxicol Pharmacol 64(2): 205-224.

Takahashi O, Oishi S. 2000. Disposition of orally administered 2,2-Bis(4-hydroxyphenyl)propane (Bisphenol A) in pregnant rats and the placental transfer to fetuses. Environ Health Perspect 108(10): 931-935.

Takahashi K, Yokota S, Tatsumi N, Fukami T, Yokoi T, Nakajima M. 2013. Cigarette smoking substantially alters plasma microRNA profiles in healthy subjects. Toxicology and applied pharmacology 272(1): 154-160.

Takamizawa J, Konishi H, Yanagisawa K, Tomida S, Osada H, Endoh H, et al. 2004. Reduced expression of the let-7 microRNAs in human lung cancers in association with shortened postoperative survival. Cancer Res 64(11): 3753-3756.

Thum T, Galuppo P, Wolf C, Fiedler J, Kneitz S, van Laake LW, et al. 2007. MicroRNAs in the human heart: a clue to fetal gene reprogramming in heart failure. Circulation 116(3): 258-267.

Tilghman SL, Bratton MR, Segar HC, Martin EC, Rhodes LV, Li M, et al. 2012. Endocrine disruptor regulation of microRNA expression in breast carcinoma cells. PLoS One 7(3): e32754.

Tseng CH. 2008. Cardiovascular disease in arsenic-exposed subjects living in the arseniasis-hyperendemic areas in Taiwan. Atherosclerosis 199(1): 12-18.

Tsukimori K, Tokunaga S, Shibata S, Uchi H, Nakayama D, Ishimaru T, et al. 2008. Long-term effects of polychlorinated biphenyls and dioxins on pregnancy outcomes in women affected by the Yusho incident. Environ Health Perspect 116(5): 626-630.

Van Pottelberge GR, Mestdagh P, Bracke KR, Thas O, van Durme YM, Joos GF, et al. 2011. MicroRNA expression in induced sputum of smokers and patients with chronic obstructive pulmonary disease. American journal of respiratory and critical care medicine 183(7): 898-906.

van Rooij E, Sutherland LB, Qi X, Richardson JA, Hill J, Olson EN. 2007. Control of stress-dependent cardiac growth and gene expression by a microRNA. Science 316(5824): 575-579.

Vasudevan S, Tong Y, Steitz JA. 2007. Switching from repression to activation: microRNAs can up-regulate translation. Science 318(5858): 1931-1934.

Voellenkle C, van Rooij J, Cappuzzello C, Greco S, Arcelli D, Di Vito L, et al. 2010. MicroRNA signatures in peripheral blood mononuclear cells of chronic heart failure patients. Physiological genomics 42(3): 420-426.

Vogt M, Munding J, Gruner M, Liffers ST, Verdoodt B, Hauk J, et al. 2011. Frequent concomitant inactivation of miR-34a and miR-34b/c by CpG methylation in colorectal, pancreatic, mammary, ovarian, urothelial, and renal cell carcinomas and soft tissue sarcomas. Virchows Arch 458(3): 313-322.

Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, et al. 2006. A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci U S A 103(7): 2257-2261.

Waliszewski SM, Aguirre AA, Infanzon RM, Silva CS, Siliceo J. 2001. Organochlorine pesticide levels in maternal adipose tissue, maternal blood serum, umbilical blood serum, and milk from inhabitants of Veracruz, Mexico. Arch Environ Contam Toxicol 40(3): 432-438.

Wang C, Yang C, Chen X, Yao B, Yang C, Zhu C, et al. 2011. Altered profile of seminal plasma microRNAs in the molecular diagnosis of male infertility. Clin Chem 57(12): 1722-1731.

Wang H, Zhang P, Chen W, Feng D, Jia Y, Xie L. 2012. Serum microRNA signatures identified by Solexa sequencing predict sepsis patients' mortality: a prospective observational study. PLoS One 7(6): e38885.

Wang X, Ha T, Liu L, Zou J, Zhang X, Kalbfleisch J, et al. 2013. Increased expression of microRNA-146a decreases myocardial ischaemia/reperfusion injury. Cardiovasc Res 97(3): 432-442.

Wang Y, Li Z, He C, Wang D, Yuan X, Chen J, et al. 2010. MicroRNAs expression signatures are associated with lineage and survival in acute leukemias. Blood Cells Mol Dis 44(3): 191-197.

Wang F, Liu W, Jin Y, Wang F, Ma J. 2014. Prenatal and neonatal exposure to perfluorooctane sulfonic acid results in aberrant changes in miRNA expression profile and levels in developing rat livers. Environ Toxicol.

Wang J, Zhang Y, Zhang W, Jin Y, Dai J. 2012. Association of perfluorooctanoic acid with HDL cholesterol and circulating miR-26b and miR-199-3p in workers of a fluorochemical plant and nearby residents. Environmental science & technology 46(17): 9274-9281.

Weigelt K, Bergink V, Burgerhout KM, Pescatori M, Wijkhuijs A, Drexhage HA. 2013. Down-regulation of inflammation-protective microRNAs 146a and 212 in monocytes of patients with postpartum psychosis. Brain Behav Immun 29: 147-155.

White NM, Bao TT, Grigull J, Youssef YM, Girgis A, Diamandis M, et al. 2011. miRNA profiling for clear cell renal cell carcinoma: biomarker discovery and identification of potential controls and consequences of miRNA dysregulation. J Urol 186(3): 1077-1083.

Wild C. 2005. Complementing the genome with an "exposome": the outstanding challenge of environmental exposure measurement in molecular epidemiology. Cancer epidemiology, biomarkers & prevention 14 (8): 1847–50.

Williams AH, Valdez G, Moresi V, Qi X, McAnally J, Elliott JL, et al. 2009. MicroRNA-206 delays ALS progression and promotes regeneration of neuromuscular synapses in mice. Science 326(5959): 1549-1554.

Wong KY, Liang R, So CC, Jin DY, Costello JF, Chim CS. 2011. Epigenetic silencing of MIR203 in multiple myeloma. British journal of haematology 154(5): 569-578.

Wu ZS, Wu Q, Wang CQ, Wang XN, Huang J, Zhao JJ, et al. 2011. miR-340 inhibition of breast cancer cell migration and invasion through targeting of oncoprotein c-Met. Cancer 117(13): 2842-2852.

Wu J, Yang T, Li X, Yang Q, Liu R, Huang J, et al. 2013. Alteration of serum miR-206 and miR-133b is associated with lung carcinogenesis induced by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. Toxicol Appl Pharmacol 267(3): 238-246.

Xi S, Yang M, Tao Y, Xu H, Shan J, Inchauste S, et al. 2010. Cigarette smoke induces C/EBP-beta-mediated activation of miR-31 in normal human respiratory epithelia and lung cancer cells. PLoS One 5(10): e13764.

Xiao C, Calado DP, Galler G, Thai TH, Patterson HC, Wang J, et al. 2007. MiR-150 controls B cell differentiation by targeting the transcription factor c-Myb. Cell 131(1): 146-159.

Xu CC, Han WQ, Xiao B, Li NN, Zhu DL, Gao PJ 2008. Differential expression of microRNAs in the aorta of spontaneously hypertensive rats. Sheng Li Xue Bao 60(4): 553-560.

Yamagishi M, Nakano K, Miyake A, Yamochi T, Kagami Y, Tsutsumi A, et al. 2012. Polycomb-mediated loss of miR-31 activates NIK-dependent NF-kappaB pathway in adult T cell leukemia and other cancers. Cancer Cell 21(1): 121-135.

Yamamoto M, Singh A, Sava F, Pui M, Tebbutt SJ, Carlsten C. 2013. MicroRNA expression in response to controlled exposure to diesel exhaust: attenuation by the antioxidant N-acetylcysteine in a randomized crossover study. Environ Health Perspect 121(6): 670-675.

Yanaihara N, Caplen N, Bowman E, Seike M, Kumamoto K, Yi M, et al. 2006. Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. Cancer cell 9(3): 189-198.

Yi C, Wang Q, Wang L, Huang Y, Li L, Liu L, et al. 2012. MiR-663, a microRNA targeting p21(WAF1/CIP1), promotes the proliferation and tumorigenesis of nasopharyngeal carcinoma. Oncogene 31(41): 4421-4433.

Yuchuan H, Ya D, Jie Z, Jingqiu C, Yanrong L, Dongliang L, et al. 2014. Circulating miRNAs might be promising biomarkers to reflect the dynamic pathological changes in smoking-related interstitial fibrosis. Toxicology and industrial health. 30 (2): 182-191.

Zampetaki A, Kiechl S, Drozdov I, Willeit P, Mayr U, Prokopi M, et al. 2010. Plasma microRNA profiling reveals loss of endothelial miR-126 and other microRNAs in type 2 diabetes. Circ Res 107(6): 810-817.

Zhang B, Pan X. 2009. RDX induces aberrant expression of microRNAs in mouse brain and liver. Environ Health Perspect 117(2): 231-240.

Zhang Y, Pan T, Zhong X, Cheng C. 2014. Nicotine upregulates microRNA-21 and promotes TGF-beta-dependent epithelial-mesenchymal transition of esophageal cancer cells. Tumour Biol.

Zhang JX, Song W, Chen ZH, Wei JH, Liao Y, Lei J et al. 2013. Prognostic and predictive value of a microRNA signature in stage II colon cancer: a microRNA expression analysis. Lancet Oncol 14(13): 1295-1306.

Zhang Y, Yan LX, Wu QN, Du ZM, Chen J, Liao DZ, et al. 2011. miR-125b is methylated and functions as a tumor suppressor by regulating the ETS1 proto-oncogene in human invasive breast cancer. Cancer Res 71(10): 3552-3562.

**Table 1.** MicroRNAs responsive to personal or environmental exposure and their role in human disease.

| miRNA | Reg. | Exposure | Disease | Source |
|---|---|---|---|---|
| Let-7e | down | TCDD | HCC, lung, pituitary & breast cancer, GEP tumors | Feitelson and Lee 2007; Qian et al. 2009; Rahman et al. 2009; Sakurai et al. 2012; Takamizawa et al. 2004 |
| | up | RDX | heart failure, asthma | Polikepahad et al. 2010; Thum et al. 2007 |
| Let-7g | down | BPA, PM | lung carcinoma, GEP tumors, breast cancer | Rahman et al. 2009; Sakurai et al. 2012 |
| miR-9 | down | PM | brain cancer, Huntingon's disease | Ferretti et al. 2009; Lau and de Strooper 2010 |
| | up | aluminum | Hodgkin lymphoma, breast cancer | Leucci et al. 2012; Ma et al. 2010 |
| miR-10b | down | formaldehyde, PM | gastric cancer | Kim K et al. 2011 |
| miR-21 | down | smoking | diabetes type 2 | Zampetaki et al., 2010 |
| | up | DEP, metal rich PM | breast cancer, glioblastoma, neo-intimal lesions, cardiac hypertrophy, atherosclerosis | Ji et al 2007; Raitoharju et al. 2011; van Rooij et al. 2007; Volinia et al. 2006 |
| miR-26b | down | DEP, BPA, PFOA | schizophrenia, CRC, breast cancer | Earle et al. 2010; Liu et al. 2011; Perkins et al. 2007 |
| miR-31 | down | DEP, TCDD | medulloblastoma, T cell leukemia | Ferretti et al. 2009; Yamagishi et al. 2012 |
| miR-34b | down | smoking (2X) | CRC, pancreatic, mammary, ovarian and renal cell carcinoma | Vogt et al. 2011 |
| miR-92b | down | smoking, DDT | medulloblastoma | Genovesi et al. 2011 |
| miR-122 | down | smoking | HCC | Bai et al. 2009 |
| | up | TCDD | hepatitis C, renal cell carcinoma, male infertility, sepsis, hyperlipidemia | Gao et al. 2012; Henke et al. 2008; Wang C et al. 2011; Wang H et al. 2012; White et al. 2011 |
| miR-125b | down | smoking (2X) | breast cancer, head and neck cancer | Nakanishi et al. 2013; Zhang et al. 2011 |
| | up | aluminum sulfate (2X) | endometriosis, cardiac hypertrophy, Alzheimer's disease | Busk and Cirera 2010; Lukiw and Alexandrov 2012; Ohlsson Teague et al. 2009 |
| miR-135b | down | DEP | medulloblastoma | Lv et al. 2012 |
| | up | smoking | CRC | Nagel et al. 2008 |
| miR-142 | down | formaldehyde | heart failure | Voellenkle et al. 2010 |
| | up | smoking | B cell ALL | Ju et al. 2009 |
| miR-143 | up | PM, ozone | colon cancer | Zhang et al. 2013 |
| miR-146a | down | smoking | postpartum psychosis, type 2 diabetes | Weigelt et al. 2013; Zampetaki et al. 2010 |
| | up | BPA, aluminum sulfate (2X) | Alzheimer's disease, Creutzfeldt Jacob disease, atherosclerosis, leukemia, protection against myocardial injury | Lukiw and Alexandrov 2012; Lukiw et al. 2011; Raitoharju et al. 2011; Wang et al. 2013; Wang Y et al. 2010 |
| miR-149 | up | BPA, DDT | melanoma | Jin et al. 2011 |
| miR-155 | down | PM | hypertension | Xu et al. 2008 |
| | up | PM | breast cancer, Hodgkin lymphoma, B-ALL | Chang et al. 2011; Kong et al. 2014; Palma et al. 2014 |

| miRNA | Reg. | Exposure | Disease | Source |
|---|---|---|---|---|
| miR-181a | down | formaldehyde | leukemia, glioma, NSCLC, breast cancer, metabolic syndrome and CAD | Gao et al. 2010; Hulsmans et al. 2012; Marcucci et al. 2008; Ota et al. 2011; Shi et al. 2008 |
| | up | TCDD | severe pre-eclampsia, male infertility | Hu et al. 2009; Wang C et al. 2011 |
| miR-203 | down | smoking, formaldehyde | myeloma | Wong et al. 2011 |
| miR-205 | up | smoking (2x) | heart failure, lung cancer | Thum et al 2007; Yanaihara et al. 2006 |
| miR-206 | up | smoking, RDX | myocardial infarct, slows ALS progression, myotonic dystrophy | Gambardella et al. 2010; Shan et al. 2009; Williams et al. 2009 |
| miR-222 | up | metal-rich PM, BPA | severe pre-eclampsia, thyroid carcinoma, prostate cancer, breast cancer | Hu et al. 2009; Miller et al. 2008; Pallante et al. 2006 |
| miR-223 | down | smoking | AML | Eyholzer et al. 2010 |
| | up | smoking | heart failure, atherosclerosis | Greco et al. 2012; Kin et al. 2012 |
| miR-338-5p | down | formaldehyde | melanoma | Caramuta et al. 2010 |
| | up | DEP | oral carcinoma | Scapoli et al. 2010 |
| miR-340 | down | Smoking | | |
| | up | Smoking | heart failure, breast cancer | Thum et al. 2007; Wu et al. 2011 |
| miR-638 | up | BPA, DDT, arsenic | lupus nephritis | Dai et al. 2009 |
| miR-663 | up | BPA, DDT, arsenic | CTCL, nasopharyngeal carcinoma, burns | Liang et al. 2012; Ralfkiaer et al. 2011; Yi et al. 2012 |

HCC: hepatocellular carcinoma, GEP: gastroenteropancreatic, CRC: colorectal carcinoma, NSCLC: non-small cell lung carcinoma, CAD: coronary artery disease, ALS: Amyotrophic lateral sclerosis, CTCL: cutaneous T-cell lymphoma.

**Table 2.** *In vitro* studies on the effects of smoking on differential miRNA expression.

| miRNA | miR function | Regulation | Tissue/cell type | Source |
|---|---|---|---|---|
| miR-15a | tumor suppressor | down | primary bronchial epithelial cells | Schembri et al. 2009 |
| miR-125b | targets P53, stress response | | | |
| miR-199b | oncogene activation | | | |
| miR-218 | tumor suppressor | | | |
| miR-31 | apoptosis, tumor suppressor | up | normal and cancer lung cells | Xi et al. 2010 |
| miR-21 | fatty acid synthesis, apoptosis | up | human squamous carcinoma cells | Zhang et al. 2014 |
| miR-452 | targets CDK1 | down | human alveolar macrophages | Graff et al. 2012 |

**Table 3.** *In vivo* studies on the effects of smoking on differential miRNA expression.

| miRNA | miR function | Regulation | Tissue/cell type | Source |
|---|---|---|---|---|
| miR-34b | p53 effector | down | mouse lung | Izzotti et al. 2011 |
| miR-421 | targets SMAD4, polycomb gene CBX7, ATM | | | |
| miR-450b | no validated targets | | | |
| miR-466 | no validated targets | | | |
| miR-469 | mouse miR not validated | | | |
| miR-135b | inflammation, oxidative stress | up | mouse lung | Halappanavar et al. 2013 |
| miR-206 | targets SERP1, BDNF, FOXP1 | up | rat serum | Wu J et al. 2013 |
| miR-133b | targets LAG1 and PTBP2 | | | |
| miR-20b | hypoxia | down | mouse lung and plasma | Huang et al. 2012 |
| miR-30e | targets UBC9, UBE21, MUC17 | | | |
| miR-125b | targets p53, stress response | | | |
| miR-128 | apoptosis | | | |
| let-7a | cell proliferation, angiogenesis | down | mouse lung | Izzotti et al. 2009 |
| let-7b | cell proliferation, angiogenesis | | | |
| let-7f | cell proliferation, angiogenesis | | | |
| miR-26a | transforming growth factor expression | | | |
| miR-30b | cell adhesion, stress response | | | |
| miR-30c | cell cycle, oncogene activation | | | |
| miR-34b | p53 effector | | | |
| miR-99b | apoptosis | | | |
| miR-122a | stress response | | | |
| miR-124a | stress response, cell growth and differentiation | | | |
| miR-125a | oncogene activation, ROS | | | |
| miR-125b | targets p53, stress response | | | |
| miR-140 | p53 effector | | | |
| miR-192 | oncogene activation | | | |
| miR-431 | protein repair, oncogene activation | | | |
| miR-92b | tumor suppresomiR | down | mouse serum | Yuchuan et al. 2014 |
| miR-668 | inflammation | | | |
| miR-700 | inflammation | | | |
| Let-7e | apoptosis | up | mouse serum | Yuchuan et al. 2014 |
| miR-19a | oncomIR | | | |
| miR-142 | immunology | | | |
| miR-191 | oncomiR | | | |
| miR-350 | unknown | | | |

reg: regulation.

**Table 4.** Human studies on the effects of smoking on differential miRNA expression.

| miRNA | miR function | Regulation | Tissue/cell type | Source |
|---|---|---|---|---|
| miR-16 | p53, cell cycle, JAK/STAT signaling | down | placenta | Maccani et al. 2010 |
| miR-21 | fatty acid synthesis, apoptosis | | | |
| miR-146a | inflammation, NFκß mediator | | | |
| miR-223 | immunology | up | maternal and cord blood | Herberth et al. 2013 |
| miR-129 | cell cycle regulation, apoptosis | down | spermatozoa | Marczylo et al. 2012 |
| miR-634 | inflammation | | | |
| miR-340 | cell migration and invasion | up | spermatozoa | Marczylo et al. 2012 |
| miR-365 | targets NKX2.1 | | | |
| miR-143 | cardiogenesis | down | gastric tissue | Stanitz et al. 2013 |
| miR-21 | fatty acid biosynthesis, apoptosis | up | gastric tissue | Stanitz et al. 2013 |
| Let-7c | cell proliferation, angiogenesis | down | induced sputum | Van Pottelberge et al. 2011 |
| miR-146a | inflammation, NFκß mediator | | | |
| miR-150 | hematopoeiesis | | | |
| miR-203 | DNA damage response | | | |
| miR-340 | cell migration and invasion | | | |
| miR-443 | unknown | | | |
| miR-223 | immunology | down | plasma MV | Badrnya et al. 2014 |
| miR-29b | apoptosis | up | plasma MV | Badrnya et al. 2014 |
| RNU6-2 | reference miR | | | |

**Table 5.** *In vitro* studies on air-pollution induced changes in miRNA expression.

| miRNA | miR function | Regulation | Tissue/cell type | Pollutant | Source |
|---|---|---|---|---|---|
| miR-26b | Wnt, p53, autophagy, TGF-ß | down | primary human bronchial epithelial cells | 10 µg/cm$^2$ DEP | Jardim et al. 2009 |
| miR-27a | apoptosis, ERα | | | | |
| miR-31 | apoptosis, tumor supressor | | | | |
| miR-96 | several unrelated targets | | | | |
| miR-135b | inflammation, oxidative stress | | | | |
| miR-374a | targets DICER, ATM | | | | |
| miR-513c | no validated targets | up | primary human bronchial epithelial cells | 10 µg/cm$^2$ DEP | Jardim et al. 2009 |
| miR-513b | no validated targets | | | | |
| miR-513a-5p | targets CD274, immunology | | | | |
| miR-923 | fragment of 28S RNA | | | | |
| miR-494 | targets PTEN | | | | |
| miR-338-5p | ABC transporters, endocytosis | | | | |
| miR-10b | angiogenesis | down | human A549 lung carcinoma cell line | 1 ppm $CH_2O$ | Rager et al. 2011 |
| miR-181a | apoptosis, oncomiR | | | | |
| miR-330 | targets E2F1, VEGFa, NTRK3 | | | | |
| miR-338-5p | ABC transporters, endocytosis | | | | |
| miR-375 | immunology | up | human bronchial epithelial cells | 3 µg/cm$^2$ DEP | Bleck et al. 2013 |
| miR-149 | immunology | down | monkey airway epithelial cells | ozone | Clay et al. 2014 |
| miR-128 | apoptosis | up | human A549 lung carcinoma cell line | $PM_{10}$ | Motta et al. 2013 |

DEP: diesel exhaust particles, $CH_2O$: formaldehyde.

**Table 6.** Human studies on air-pollution induced changes in miRNA expression.

| miRNA | miR function | Regulation | Tissue/cell type | Pollutant | Source |
|---|---|---|---|---|---|
| miR-21 | fatty acid synthesis, apoptosis | up | peripheral blood | 300 µg $PM_{2.5}/m^3$ DEP | Yamamoto et al. 2013 |
| miR-30e | targets UBC9, MUC17 | | | | |
| miR-144 | targets Klfd, FGG, PLAG1 | | | | |
| miR-215 | cell cycle, p53 activation | | | | |
| miR-21 | fatty acid synthesis, apoptosis | up | blood leukocytes | metal-rich PM | Bollati et al. 2010 |
| miR-222 | cell cycle regulation | | | | |
| miR-375 | Immunology | up | bronchial epithelial cells | 3 µg/$cm^2$ DEP | Bleck et al. 2013 |
| miR-34a | cardiogenesis | up | gastric tissue | urban living | Stanitz et al. 2013 |
| miR-143 | cardiogenesis | | | | |
| miR-10b | angiogenesis | up | spermatozoa | metal-rich PM | Li et al. 2012a |
| miR-33b | lipid metabolism | | | | |
| miR-106a | oncomiR | | | | |
| miR-155 | inflammation | | | | |
| miR-183 | oncomiR | | | | |
| miR-205 | oncomiR | | | | |
| miR-208a | cardiac hypertrophy | | | | |
| miR-222 | cell cycle regulation | | | | |
| miR-223 | immunology | | | | |
| Let-7d | proliferation, angiogenesis | down | spermatozoa | metal-rich PM | Li et al. 2012a |
| miR-363 | DNA damage response | | | | |
| miR-25 | DNA damage response | up | induced sputum | ozone | Fry et al. 2014 |
| miR-132 | angiogenesis | | | | |
| miR-143 | cardiogenesis | | | | |
| miR-145 | tumor suppressor | | | | |
| miR-199a | oncogene activation | | | | |
| miR-199b | oncogene activation | | | | |
| miR-222 | cell cycle regulation | | | | |
| miR-223 | immunology | | | | |
| miR-424 | angiogenesis | | | | |
| miR-582 | anti-apoptosis | | | | |
| miR-1 | apoptosis | down | leukocytes | $PM_{2.5}$; BC; OC; $SO_4^{2-}$ | Fossati et al. 2014 |
| miR-9 | neuronal differentiation | | | | |
| miR-21 | fatty acid synthesis, apoptosis | | | | |
| miR-126 | angiogenesis | | | | |
| miR-135a | inflammation | | | | |
| miR-146a | inflammation, NFκß mediator | | | | |
| miR-155 | inflammation | | | | |
| miR-222 | cell cycle regulation | | | | |
| miR-128 | apoptosis | up | plasma microvesicles | $PM_{10}$ | Motta et al. 2013 |

DEP: diesel exhaust particles, PM: particulate matter, BC: black carbon, OC: organic carbon.

**Table 7.** Studies on nanoparticle-induced changes in miRNA expression.

| miRNA | miR function | Regulation | Pollutant | Source |
|---|---|---|---|---|
| miR-21 | fatty acid synthesis, apoptosis | up | 0.268 or 0.162 mg carbon black NP | Bourdon et al. 2012 |
| miR-135b | inflammation, oxidative stress | | | |
| miR-146 | inflammation, NFκß mediator | | | |
| miR-122 | stress response | up | 70 nm in silica NP | Nagano et al. 2013 |
| miR-192 | oncogene activation | | | |
| Let-7a | cell proliferation, angiogenesis | up | 100 nm gold NP | Balansky et al. 2013 |
| miR-183 | oncomiR | | | |

NP: nanoparticles.

**Table 8.** *In vitro* studies on chemically induced changes in miRNA expression.

| miRNA | miR function | Regulation | Tissue/cell type | Chemical | Source |
|---|---|---|---|---|---|
| let-7g | cell proliferation, angiogenesis | down | MCF-7 cells | BPA | Tilghman et al. 2012 |
| let-7f | cell proliferation, angiogenesis | | | | |
| miR-21 | fatty acid biosynthesis, apoptosis | | | | |
| miR-26b | Wnt, p53, autophagy, TGF-ß | | | | |
| miR-342-3p | tumor suppressomiR | | | | |
| miR-15b | tumor suppressor targeting BCL2 | down | MCF-7 cells | BPA/DDT | Tilghman et al. 2012 |
| miR-222 | cell cycle regulation | up | MCF-7 cells | BPA | Tilghman et al. 2012 |
| miR-638 | no known function | up | MCF-7 cells | BPA/DDT | Tilghman et al. 2012 |
| miR-663 | immunology, oxidative stress | down | MCF-7 cells | DDT | Tilghman et al. 2012 |
| miR-1915 | no known function | | | | |
| miR-27b | angiogenesis | | | | |
| miR-92a | tumor supressomiR | | | | |
| miR-92b | tumor supressomiR | | | | |
| miR-1308 | no known function | up | MCF-7 cells | DDT | Tilghman et al. 2012 |
| miR-146a | inflammation, NFκß mediator | up | human placental cell lines | BPA | Avissar-Whiting et al. 2010 |
| miR-150 | hematopoeiesis | down | Jurkat T cell line | arsenic | Sturchio et al. 2014 |
| miR-30d | autophagy | up | Jurkat T cell line | arsenic | Sturchio et al. 2014 |
| miR-142 | immunology | | | | |
| miR-181a | apoptosis, oncomiR | | | | |
| miR-221 | DNA damage response | | | | |
| miR-222 | cell cycle regulation | | | | |
| miR-638 | no known function | | | | |
| miR-663 | immunology, oxidative stress | | | | |
| miR-190 | oncomiR | up | human bronchial epithelial cells | arsenic | Beezhold et al. 2011 |
| miR-19b | oncomiR | up | HUVEC cells | arsenic | Li et al. 2012b |
| miR-21 | fatty acid biosynthesis, apoptosis | | | | |
| miR-24 | oncomiR | | | | |
| miR-29b | apoptosis | | | | |
| miR-33a | lipid metabolism | | | | |
| miR-198 | cell proliferation | | | | |
| miR-508-5p | cell invasion and migration | | | | |
| miR-1252 | no known function | | | | |
| miR-181a | apoptosis, oncomiR | up | HepG2 cells | PAH | Song et al. 2013 |
| miR-181b | apoptosis, oncomiR | | | | |
| miR-181d | apoptosis, oncomiR | | | | |

**Table 9.** *In vivo* studies on chemically induced changes in miRNA expression.

| miRNA | miR function | Regulation | Tissue/cell type | Chemical | Source |
|---|---|---|---|---|---|
| let-7e | apoptosis | down | fetal mouse thymocytes | TCDD | Singh et al. 2012 |
| miR-18b | apoptosis | | | | |
| miR-23a | apoptosis | | | | |
| miR-23b | apoptosis | | | | |
| miR-27a | apoptosis, ERα | | | | |
| miR-28 | apoptosis | | | | |
| miR-29a | apoptosis | | | | |
| miR-31 | apoptosis, tumor supressomiR | | | | |
| miR-98 | apoptosis | | | | |
| miR-101b | apoptosis | | | | |
| miR-181c | apoptosis, oncomiR | | | | |
| miR-182 | apoptosis | | | | |
| miR-200a | apoptosis, cell cycle, MAPK | | | | |
| miR-23 | apoptosis | | | | |
| miR-290 | apoptosis | | | | |
| miR-335 | apoptosis | | | | |
| miR-491 | apoptosis, targets BCL-XL | | | | |
| miR-122 | stress response | up | fetal mouse thymocytes | TCDD | Singh et al. 2012 |
| miR-181a | oncomiR | | | | |
| miR-125b | targets P53, stress response | up | monkey nasal epithelium | formaldehyde | Rager et al. 2013 |
| miR-152 | tumor suppressor, methylation | | | | |
| miR-219 | NMDA receptor signaling | | | | |
| miR-532 | unknown | | | | |
| miR-22 | PTEN, AKT signaling | down | monkey nasal epithelium | formaldehyde | Rager et al. 2013 |
| miR-26b | Wnt, p53, autophagy, TGF-ß | | | | |
| miR-29a | apoptosis | | | | |
| miR-140 | p53 effector | | | | |
| miR-142 | Immunology | | | | |
| miR-145 | tumor suppressor, stem cell different | | | | |
| miR-203 | DNA damage response | | | | |
| miR-374a | targets DICER, ATM | | | | |
| miR-520f | Unknown | | | | |
| miR-27a | apoptosis, ERα | down | mouse brain and liver | RDX | Zhang and Pan 2009 |
| miR-200c | Apoptosis | | | | |
| let7-e | Apoptosis | | | | |
| miR-206 | targets SERP1, BDNF, FOXP1 | | | | |
| miR-451 | targets PI3K/AKT | down | rat liver | PFOS | Wang et al. 2014 |

| miRNA | miR function | Regulation | Tissue/cell type | Chemical | Source |
|---|---|---|---|---|---|
| miR-23a | Apoptosis | up | rat liver | PFOS | Wang et al. 2014 |
| miR-25 | DNA damage response | | | | |
| miR-125a | oncogene activation, ROS | | | | |
| miR-133a | smooth muscle differentiation | | | | |
| miR-133b | targets LAG1 and PTBP2 | | | | |
| miR-206 | targets SERP1, BDNF, FOXP1 | | | | |
| miR-494 | targets PTEN | | | | |
| miR-542 | DNA damage response | | | | |

**Table 10.** human studies on chemically induced changes in miRNA expression.

| miRNA | miR function | Regulation | Tissue/cell type | Chemical | Source |
|---|---|---|---|---|---|
| miR-191 | oncomiR | up | peripheral blood | PCB-169 | Guida et al. 2013 |
| miR-146a | inflammation, NFκß mediator | up | fetal brain cells | aluminum | Pogue et al. 2009 |
| miR-9 | neuronal differentiation | | | | |
| miR-125b | targets p53, stress response | up | fetal brain cells | aluminum | Lukiw and Pogue, 2007 |
| miR-128 | apoptosis | | | | |
| miR-199a | oncogene activation | up | serum | PFOA | Wang J et al. 2012 |
| miR-21 | fatty acid biosynthesis,apoptosis | up | blood samples | arsenic | Kong et al. 2012 |
| miR-26b | Wnt, p53, autophagy, TGF-ß | | | | |
| Let-7a | cell proliferation, angiogenesis | up | cord blood | arsenic | Rager et al. 2014 |
| miR-16 | p53, cell cycle, JAK/STAT | | | | |
| miR-17 | DNA damage response | | | | |
| miR-20a | angiogenesis | | | | |
| miR-20b | hypoxia | | | | |
| miR-26b | Wnt, p53, autophagy, TGF-ß | | | | |
| miR-96 | several unrelated targets | | | | |
| miR-98 | apoptosis | | | | |
| miR-107 | targets Notch2 | | | | |
| miR-126 | angiogenesis | | | | |
| miR-195 | tumor suppresomiR | | | | |
| miR-454 | unknown | | | | |
| miR-24 | oncomiR | down | plasma | PAH | Deng et al., 2014 |
| miR-27a | apoptosis, ERα | | | | |
| miR-28 | aApoptosis | | | | |
| miR-142 | immunology | | | | |
| miR-150 | hematopoeiesis | up | plasma | PAH | Deng et al., 2014 |

## Figure Legends

**Figure 1.** Overview of miRNA biogenesis. The canonical maturation of a miRNA includes the production of the primary miRNA transcript (pri-miRNA) by RNA polymerase II or III and cleavage of the pri-miRNA by the microprocessor complex Drosha–DGCR8 (Pasha) in the nucleus. The resulting precursor hairpin, the pre-miRNA, is exported from the nucleus by Exportin-5–Ran-GTP. In the cytoplasm, the RNase Dicer in complex with the double-stranded RNA-binding protein TRBP cleaves the pre-miRNA hairpin to its mature length. The functional strand of the mature miRNA is loaded together with Argonaute (Ago2) proteins into the RNA-induced silencing complex (RISC), where it guides RISC to silence target mRNAs through mRNA cleavage, translational repression or deadenylation, whereas the passenger strand (black) is degraded.

**Figure 2.** Flowchart of included studies.

**Figure 3.** Venn diagram displaying common and distinct microRNAs associated with smoking from *in vitro*, *in vivo* and human studies. Those miRNAs listed in bold were identified in more than one study included in this meta-analysis.

**Figure 4.** Venn diagram displaying common and distinct microRNAs associated with air pollution exposure from *in vitro* and human studies. Those miRNAs listed in bold were identified in more than one study included in this meta-analysis.

**Figure 5.** Venn diagram displaying common and distinct microRNAs associated with arsenic exposure from *in vitro* and human studies. Those miRNAs listed in bold were identified in more than one study included in this meta-analysis.

Figure 1.

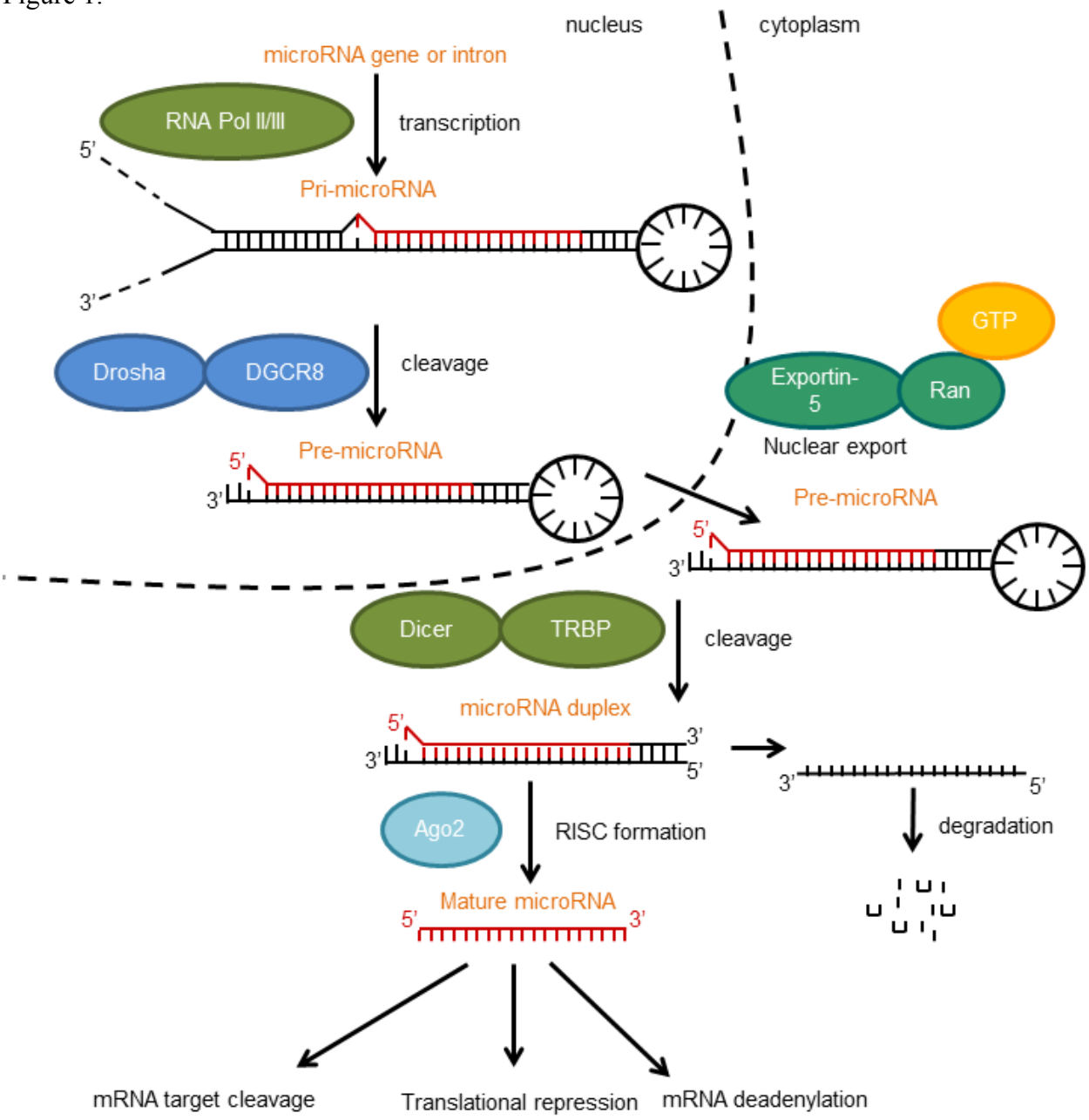

nucleus
cytoplasm
microRNA gene or intron
RNA Pol II/III
transcription
5'
Pri-microRNA
3'
Drosha
DGCR8
cleavage
GTP
Exportin-5
Ran
Nuclear export
5'
Pre-microRNA
3'
Pre-microRNA
5'
3'
Dicer
TRBP
cleavage
microRNA duplex
5'
3'
3'
5'
3'
5'
degradation
Ago2
RISC formation
Mature microRNA
5'
3'
mRNA target cleavage
Translational repression
mRNA deadenylation

Figure 2.

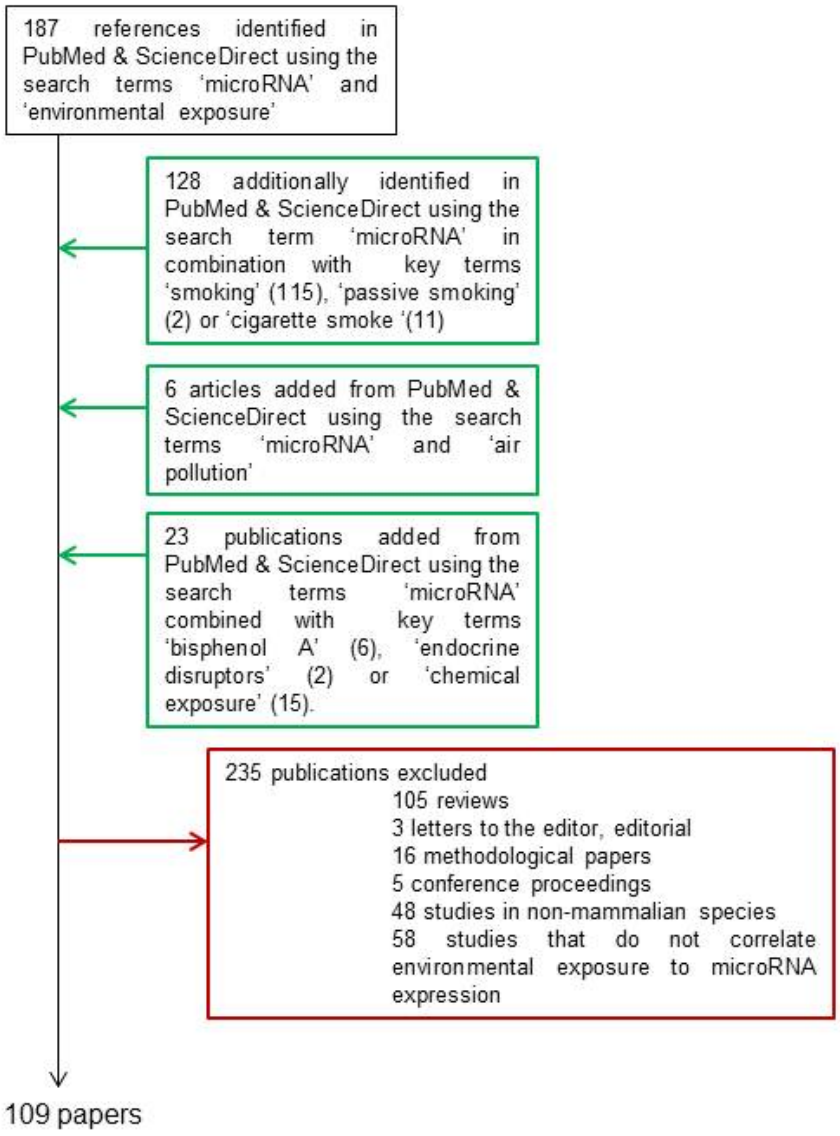

187 references identified in PubMed & ScienceDirect using the search terms 'microRNA' and 'environmental exposure'
128 additionally identified in PubMed & ScienceDirect using the search term 'microRNA' in combination with key terms 'smoking' (115), 'passive smoking' (2) or 'cigarette smoke '(11)
6 articles added from PubMed & ScienceDirect using the search terms 'microRNA' and 'air pollution'
23 publications added from PubMed & ScienceDirect using the search terms 'microRNA' combined with key terms 'bisphenol A' (6), 'endocrine disruptors' (2) or 'chemical exposure' (15).
235 publications excluded
105 reviews
3 letters to the editor, editorial
16 methodological papers
5 conference proceedings
48 studies in non-mammalian species
58 studies that do not correlate environmental exposure to microRNA expression
109 papers

Figure 3.

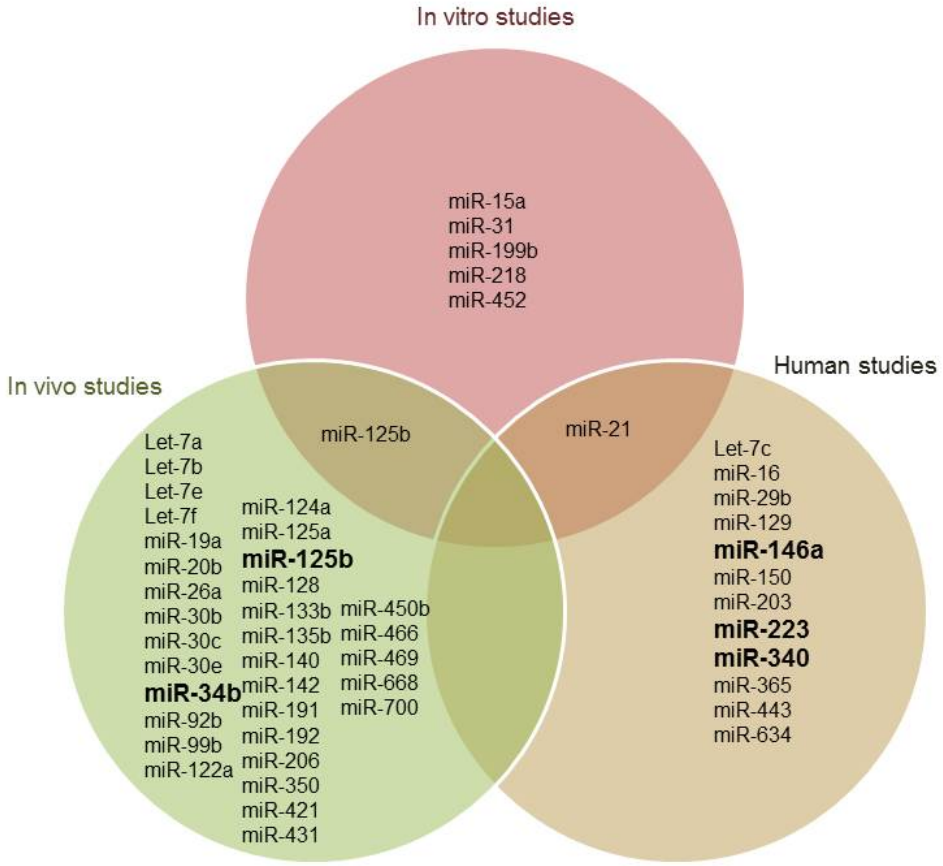

In vitro studies
miR-15a
miR-31
miR-199b
miR-218
miR-452
In vivo studies
Human studies
miR-125b
miR-21
Let-7a
Let-7b
Let-7e
Let-7f
miR-19a
miR-20b
miR-26a
miR-30b
miR-30c
miR-30e
miR-34b
miR-92b
miR-99b
miR-122a
miR-124a
miR-125a
miR-125b
miR-128
miR-133b
miR-135b
miR-140
miR-142
miR-191
miR-192
miR-206
miR-350
miR-421
miR-431
miR-450b
miR-466
miR-469
miR-668
miR-700
Let-7c
miR-16
miR-29b
miR-129
miR-146a
miR-150
miR-203
miR-223
miR-340
miR-365
miR-443
miR-634

Figure 4.

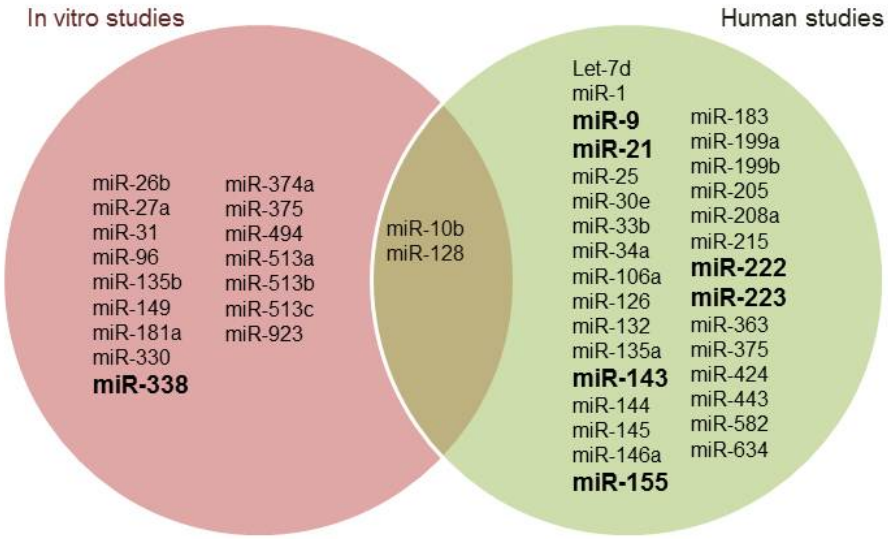
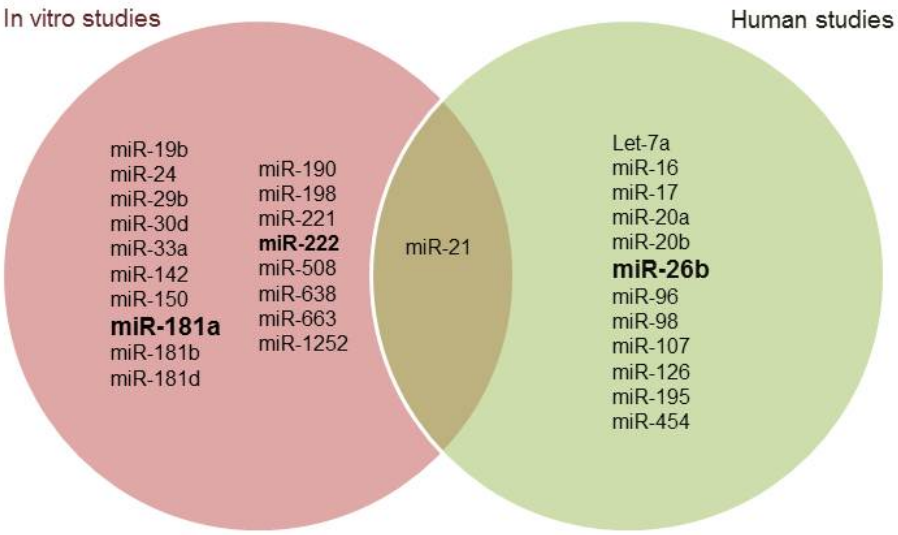

In vitro studies
Human studies
miR-26b
miR-27a
miR-31
miR-96
miR-135b
miR-149
miR-181a
miR-330
miR-338
miR-374a
miR-375
miR-494
miR-513a
miR-513b
miR-513c
miR-923
miR-10b
miR-128
Let-7d
miR-1
miR-9
miR-21
miR-25
miR-30e
miR-33b
miR-34a
miR-106a
miR-126
miR-132
miR-135a
miR-143
miR-144
miR-145
miR-146a
miR-155
miR-183
miR-199a
miR-199b
miR-205
miR-208a
miR-215
miR-222
miR-223
miR-363
miR-375
miR-424
miR-443
miR-582
miR-634

Figure 5.


In vitro studies
Human studies
miR-19b
miR-24
miR-29b
miR-30d
miR-33a
miR-142
miR-150
miR-181a
miR-181b
miR-181d
miR-190
miR-198
miR-221
miR-222
miR-508
miR-638
miR-663
miR-1252
miR-21
Let-7a
miR-16
miR-17
miR-20a
miR-20b
miR-26b
miR-96
miR-98
miR-107
miR-126
miR-195
miR-454